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EDITORIAL

PAGET'S DISEASE

This is a mysterious disease, the etiology of which has not been discovered. In 1876, at a time when bone diseases were not well understood, Sir James Paget* read a paper entitled "On a Form of Chronic Inflammation of Bones" before the Royal Medical Chirurgical Society of London. His description has not been improved upon by anyone. He stated:

> The disease begins in middle age or later, is very slow in progress, may continue for many years without influence on general health, and may give no other trouble than those which are due to changes of shape, size, and direction of the diseased bones. Even when the skull is hugely thickened, and all its bones exceedingly altered in structure, the mind remains unaffected. The disease affects most frequently the long bones of the lower extremities and the skull, and is usually symmetrical. The bones enlarge and soften, and these bearing weight yield and become unnaturally curved and misshapen. The spine, whether by yielding to the weight of the overgrown skull or by change in its own structure, may sink and seem to shorten with greatly increased dorsal and lumbar curves; the pelvis may become wide; the necks of the femora may become nearly horizontal, but the limbs, however misshapen, remain strong and fit to support the trunks.

Today the diagnostician has the advantage of additional help from the study of the blood chemistry, since in Paget's disease the phosphatase level is unusually high.

The histologic findings are quite characteristic; if the jaws are involved, a biopsy in addition to the phosphatase determination helps in the differential diagnosis from the more common types of fibro-osseous dysplasia, both the polyostotic and the monostotic types.

It is generally believed that the maxilla is involved more often than the mandible. We are presenting in this issue papers which deal with Paget's disease of the jaws and its effect on the involved teeth.

Complications are loss of hearing and sight due to obliteration of the foramina through which the nerves emerge. Impingement of the second and

*Paget, J.: On a Form of Chronic Inflammation of Bones (Osteitis deformans), Medico-Chirurgical Tr., London, 1877.

third divisions of the fifth nerve may account for neuralgic pains. The electric tests of teeth involved may be found negative. Cahn* has reported the case of a woman suffering from tic douloureux.

It has been known that osteogenic sarcoma may develop in bones affected by the disease. Kienbock and Selka** reported a case with multiple bone sarcoma. The contributions in this issue include reports of a case in which a giant-cell tumor developed in a maxilla affected by Paget's disease and another case with an osteogenic sarcoma. These are two rare cases of unusual interest to oral surgeons.

K. H. T.

*Cahn, L. R.: Pathology of the Oral Cavity, Baltimore, 1941, Williams & Wilkins Company, p.75.

**Kienbock, R., and Selka, A.: Ein Fall von Paget's Knochenkrankheit mit multiplen Sarkomen der Knochen, Beitr. z. klin. Chir. 162: 246, 1935.

OPERATIVE ORAL SURGERY

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A REVIEW OF THE DIFFERENTIAL DIAGNOSIS OF ORAL MANIFESTATIONS IN EARLY OSTEITIS DEFORMANS

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Out of the disordered chaos which represented nineteenth century disease nomenclature, Sir James Paget, in 1867, selected a specific group of symptoms and findings and classified them into a single disease entity which still bears his name. Because of its eventual tendency toward gross distortion of the skeletal frame, this entity is also, and more accurately, termed osteitis deformans. Even this name is not completely accurate, however, since the disease is not a true inflammation but, rather, a dystrophy.

Paget's disease is a chronic, progressive disturbance in bone metabolism characterized by an initial phase of deossification and softening, followed by a bizarre, dysplastic type of reossification not related to functional requirements. The two processes may take place simultaneously, or they may alternate. In the early phases of the disease resorption is usually more prominent, but in the advanced stages a typical osteogenesis becomes increasingly pronounced, resulting in the progressive enlargement and deformity of affected bones. The course of the disease is prolonged, usually encompassing several decades, and the disease appears to involve both sexes equally. It is seldom encountered in patients under 40 years of age.

The etiology is unknown, although it has been thought to be related to hyperthyroidism, viral infection, parasitic infestation, and again recently to a congenital pattern.

Originally classified as a rare affliction, Paget's disease has a recent increased incidence paralleling the current trend toward frequent dental and medical roentgenographic studies. Meschan[2] states that approximately 3 per cent of all patients over 40 may be found to show some changes suggestive of this condition.

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The oral practitioner's chief interest lies in the fact that a number of incipient cases of Paget's disease have been discovered through their oral manifestations; moreover, Stafne and Austin[4] report that twenty-three of 138 cases of Paget's disease (one out of six) had evidence of the disease in the maxilla or the mandible.

Thus, under the supposition that a patient has presented himself with latent Paget's disease (either with the complaints listed below or for the diagnosis of an area or areas of radiolucency and/or sclerosis of the mandible or maxilla), the diagnostician should be cognizant of the following findings in the preliminary examination:

Clinical Signs and Symptoms.—Diagnosis of the early stages of the disease is difficult from a symptomatologic basis because of the insidious nature of onset. The initial presenting symptoms are obscure and usually consist of unexplained bone pain, intermittent swelling, muscle cramps, occasionally a pathologic fracture, or any combination of these. (It should be mentioned here, of course, that the symptomatology in the late stages of the disease is usually quite frank and consists of osteolytic deformities of the weight-bearing bones, resulting in kyphosis, broadened pelvis, spinal scoliosis, and bowing of the skeletal frame. As progressive softening is followed by uncontrolled recalcification of deformed bones, the patient eventually develops thickening of the calvaria, prominent supraorbital ridges, enlarged maxillary and mandibular bones, and a grotesquely enlarged cranial vault.)

Oral and Regional Physical Examination.—In the case of an early osteitis deformans, the oral and regional examination, except for the complaint of tenderness, commonly reveals nothing positive. There is no tumescence, paresthesia, loosening of teeth, or lymphadenopathy. This procedure should, however, eliminate such local factors as tooth abscesses, periodontal abscesses, granulomas, and pericoronal infections.

Initial Radiography.—We shall here assume that full-mouth apical and occlusal films are taken. These, plus paranasal sinus and lateral and posteroanterior jaw films, must surely be termed prerequisites to the diagnosis of any osteitic lesion whose identification is not immediately apparent.

While preliminary diagnosis of the affliction necessitates roentgenograms of the teeth or bones and these show characteristic changes long before deformities occur, it is quite difficult to make a positive diagnosis of early Paget's disease on this basis alone. This is because of the tendency of these radiographs to mimic a myriad of other pathologic conditions.

The early latent osteitis deformans presents itself radiographically as a radiolucent, ill-defined, demineralized area or areas, with or without regions of increased density, sclerotic bone, and cortical thickening. The lesion may be monostotic, polyostotic, or merostotic.

With those factors that are of distinctly local nature having been eliminated, the other causalgias that might produce such a roentgenographic picture must be considered.

Once the initial roentgenograms, clinical symptomatology, and oral examination have been completed, the most efficient steps required to facilitate the differential diagnosis of osteitis deformans would be as follows: adequate clinical history, complete physical examination by an internist, skull and total bone survey, repeat jaw films, blood chemistry findings, and biopsy.

An adequate clinical history would suggest the routine elimination of such osteolytic conditions as rickets, osteomalacia, disuse osteoporosis, senile osteoporosis (always associated with other evidences of senescence), postmenopausal osteoporosis, parathyroid administration, and overdosage of vitamin D.

General physical examination would readily distinguish those osteolytic diseases which evidence gross systemic pathologic findings. This would include sprue, Boeck's sarcoid (osteitis tuberculosa multiplex), pancreatic steatorrhea, Cushings's syndrome, acute osteomyelitis, carcinomatosis, sarcomatosis, and lipoid storage diseases (Gaucher's, Hand-Schüller-Christian, and Niemann-Pick).

Skull and total bone survey comprises the third step. Although it is not a generalized disease of bone and, unlike osteitis fibrosa cystica, does not always involve the entire skeleton, osteitis deformans may be polyostotic, and few or many bones may be involved. Thus, a primary rule in the radiology of bone disease is invoked, namely, whenever a bone disease of undetermined etiology is identified, it is important to obtain a complete skeletal survey, involving not only the extremities but the skull, thoracic cage, spine, pelvis, and lung parenchyma as well. Early skull involvement more commonly manifests itself by the appearance of multiple confluent, maplike resorptions of bone called osteoporosis circumscripta and by a bizarre deposition of bone in irregular, somewhat circular areas of increased density giving rise to the "cotton wool" appearance so characteristic of Paget's disease. Loss of distinction between the tables and the diploë is also seen.

Where other skeletal distribution occurs, it presents a similar picture with elongated or rhomboid demineralization of cancellous bone and thickening of the cortex and replacement of old structures by new ones of irregular trabeculation. As a rule, only part of the bone is afflicted.

Repeat jaw films with a re-evaluation of findings would then be taken with the following comparative radiographic description of Paget's disease in mind.

In the maxilla and mandible the area of radiolucency is ill defined and not encapsulated. The demineralized regions usually appear as coarse, irregular, osteoporotic spongiosa, with osteosclerotic trabecular disorganization. There are intermediate areas of porotic medullary bone due to fibrosis, and around these there is an exfoliating sclerotic cortex. (The appearance of osteosclerosis may be a relative matter, inasmuch as bone may sometimes be sclerotic only in regard to the surrounding osteoporosis.)

The lamellae run in all directions, and there are no Haversian systems. Thus, loss of architecture is perhaps the cardinal radiographic symptom. Around the tooth roots, hypercementosis is usually, but not always, present.

In view of the above description, and for the sake of simplicity, certain neoplastic lesions can be discounted immediately on the grounds that, whereas they may be osteolytic in character, their x-ray presentation simply does not resemble this picture. This group would include odontogenic cysts (including follicular and dentigerous), myxomatous cysts, odontogenic fibromas, cavernous angiomas, ameloblastomas, and central mixed tumors, all of which produce defects that are cystic or polycystic in appearance and are often multiloculated, as well as chondromas, giant-cell tumors, and myxomas, which are, generically speaking, circumscribed, solid, and well defined.

This is not to negate the importance of biopsy and blood chemistry findings in such diseases, for all lesions which fail to heal should be biopsied, and no diagnosis of bone defect should be made on the basis of roentgenographic findings alone. They are not, however, readily confused with Paget's disease.

Also readily eliminated by the diagnostician are *osteopoikilosis,* which is characterized by a speckled appearance of bone, widely disseminated, round or oval, and distinctly circumscribed spotty shadows of calcium density, and *leontiasis ossea,* which involves the base of the skull and the facial bones primarily, and has a homogenous sclerosis, no rarefaction, and no cotton wool appearance.

Of those remaining diseases whose roentgenograms commonly tend to simulate osteitis deformans, the following radiologic and physical factors should be considered:

1. *Osteogenic sarcoma** (including osteosarcoma, reticulum-cell sarcoma, Ewing's sarcoma, chondrosarcoma, etc.) generally presents periosteal formations which appear as rods or lamellae extending vertically from the bone into the edges of the tumor, giving the characteristic "sun ray" effect. There is always lack of a definite outline of demarcation of the tumor because of its invasiveness, but the deossification of bone is compensated for by the formation of new bone at the periosteal surface, away from the tumor.

2. *Multiple myeloma* (plasmacytoma) has characteristics similar to Paget's disease and, again, requires biopsy for definite diagnosis. Radiographically, the individual lesions appear more cystic, nodular, and well circumscribed. There is bone destruction but no bone formation. Most significant, however, is the fact that the lesions are multiple and are found over the entire skeleton. Affected areas, particularly the mandible, are characteristically rounded, multiple, and grape sized. They may enlarge and merge, forming oval, punched-out, or elongated rarefied distribution.

*It should be noted also that all tumors eventually present masses and often produce asymmetry, loosening of teeth, and paresthesia while osteitis deformans evidences only soft tissue swelling.

3. *Metastatic carcinomas* occur late and are usually secondary to primary tumors of the prostate, breast, lung, thyroid, parotid, gland, and lip. The appearance is usually that of spongiosclerotic localized new bone formation, and the skull may show multiple demarcated areas of sclerosis. The increase in bone density is compact and fairly regular in shape, as contrasted to the bizarre, disorganized trabeculation of Paget's disease, and multiple punched-out osteolytic areas may be seen throughout the skeleton.

4. *Primary hyperparathyroidism* (osteitis fibrosa cystica) produces a generalized type of osteoporosis or foci of osteitis fibrosa with or without cysts. In addition to a host of physical findings absent in Paget's disease, including muscular hypotonia, polydipsia, and polyuria, this disease gives an x-ray picture of diffuse osteoporosis and, if cysts are present, they are multiple, trabeculated structures with a spongy appearance. Giant-cell bone tumors may present themselves and the disease, unlike Paget's, involves the entire skeleton.

5. *Secondary or dry osteomyelitis* shows a mottled osteoporosis with maximal cortical involvement. The infectious process appears to extend irregularly in all directions from the periosteum and cortex. Sequestration is seldom seen, and very little sclerosis or evidence of new bone formation is obtained. (Clinically the cardinal signs of soft tissue inflammation often precede radiographic evidence.)

6. *Polyostotic fibrous dysplasia* usually involves many bones, but it may be monostotic. There is a substitution of the bone substance by dense fibrous connective tissue. Radiographically, there is an irregular fibrocystic appearance with coarse loculations and honeycombing, and it commonly shows more perimeter than Paget's disease. Unlike Paget's disease, polyostotic fibrous dysplasia is a disease of childhood, although the diseased areas never reossify and it may not be discovered until adult life. It is sometimes distinguished by its association with endocrine disturbances and skin pigmentation in the pattern of Albright's syndrome.

7. *Fibro-osteomas* vary greatly. They may show either radiopacity or a circumscribed radiolucency with an osteitic margin; on the other hand, they may be cystic. There are usually islands of greater density due to partly calcified osteoid tissue, and the ossifying fibrous tissue may give an irregular granular appearance. They are almost always well defined in contradistinction to osteitis deformans.

Once the differentiation has proceeded to this state, wherein a presumptive diagnosis may be approached, the next logical step should be a body fluid analysis.

A complete blood and urine work-up, with emphasis upon the results of the *blood chemistry findings,* provides a most efficeint screening technique in

the further differentiation of Paget's disease. Of these analyses, the most important finding is an elevation of the serum alkaline phosphatase level, with all other levels remaining normal. This, while not pathognomonic for the disease, is extremely significant. The normal serum alkaline phosphatase level, transmitted in Bodansky units, is 1 to 4. Rarefying osteitis deformans will always give an elevation of this level, which may vary from 6 to 135 units. Primary hyperparathyroidism with osteitis fibrosa will also show an increased serum alkaline phosphatase level, but of necessity it must concomitantly show elevated calcium and decreased inorganic phosphorous levels, whereas these fractions do not vary in osteitis deformans.

This feature distinguishes it from all other nondietary osteolytic diseases, with the exceptions of osteogenic sarcoma, osteoplastic bone metastases, and multiple myeloma. Of these, blood chemistry and urine assays may suggest the elimination of the last two, inasmuch as (1) an elevated serum acid phosphatase in addition to an elevated serum alkaline phosphatase is strongly indicative of a metastasizing carcinoma and (2) multiple myeloma shows a great increase in the blood globulin fraction, and thus the albumin-globulin ratio (normally 1.3 to 3.1) is grossly distorted. In addition to hyperglobulinemia, a hypercalcemia and the presence of Bence-Jones proteinuria are fundamental diagnostic findings in these diseases.

Biopsy is the final step to be discussed here. In the portfolio of diagnostic procedures, there is none more definitive than the tissue section, and any bone lesion whose etiolgy is not immediately apparent, regardless of appearance, demands histopathologic study.

Paget's disease has been described microscopically as having fibrous and irregularly disposed trabeculae made up of intercalated segments of bone to give a mosaic structure due to repeated destruction and new formation of bone. There are fibrosis of marrow, numerous osteoclasts, osteoblasts, fibroblasts, lymphocytes, and giant cells. Areas of osteoblastic and osteoclastic activity are usually manifested.

CASE REPORTS

CASE 1.—E. R., a 54-year-old married white male house painter, presented himself to our office in November, 1956, with a chief complaint of chronic severe pain in his lower jaw and intermittent swelling of the left side of his face which had persisted for four and one-half months. He also complained of pain in the back of his neck, with periodic episodes of limitation of motion.

He stated that he was in apparent good health until June, 1956, at which time his lower jaw began to ache in the left submental area. The patient, who was edentulous but wearing dentures, consulted his dentist, who suggested that new dentures be constructed. These were completed and fitted in August, at which time he began to notice slight left-sided trismus.

A number of denture adjustments were made without any pain relief, and the pain and intermittent swelling were equally distressing with or without the dentures. Therefore, the patient was referred to us on November 8.

History.—His past history was essentially noncontributory. He admitted having had all the childhood diseases, with the exception of scarlet fever, and denied all other diseases

categorically. He reported having had one previous hospital admission, that for a hemorrhoidectomy in 1953. A full-mouth extraction was performed in 1944 without apparent complication.

He denied any evidence of anorexia, insomnia, drug usage (except for analgesics for his present illness), or alcohol consumption, and admitted that he smoked one and one-half packs of cigarettes per day.

Fig. 1.

Fig. 2.

Figs. 1 and 2.—Case 1. Posteroanterior and lateral roentgenograms showing ill-defined zone of radiolucency in left mandible.

As for family history, the patient stated that his mother and father had died of "old age." All of the siblings (three brothers and two sisters) were living and well. The patient had no children.

Review of the systems was essentially negative, with the exception of his present illness.

Physical Examination.—Examination revealed a well-developed, well-nourished, middle-aged white man sitting upright and in no acute distress.

His face, head, and neck were symmetrical. There was no palpable lymphadenopathy, and there was no evidence of tumescence, edema, paresthesia, or pigmentation.

He had slight tenderness in the back of the neck and in the left mandibular area, both intra- and extraorally. There was slight trismus of the left temporomandibular joint, but no pain, subluxation, or crepitation.

He was edentulous and was unable to wear both prostheses at the same time because of his inability to open his mouth widely enough to accommodate both dentures.

Preliminary Occlusal, Posteroanterior, and Lateral Jaw Films.—There was a large, ill-defined zone of radiolucency in the medullary portion of the left mandible extending from the lower portion of the ascending ramus forward to beyond the submental area. The outlines of the ramus were intact. It appeared neither cystic nor encapsulated and showed several areas of faulty trabeculation. There was minimal cortical sclerosis of the outer table at the angle. The right mandibular angle had some areas of rarefaction but no true bone destruction, as there was on the left side (Figs. 1 and 2).

Differential Diagnosis.—The patient's clinical history prior to his present illness was nonrevealing. A general physical examination in September, 1956, was essentially negative.

Repeat roentgenograms, including lateral, posteroanterior jaw, and paranasal sinus films taken at Lutheran Hospital in January, 1957, revealed the following: "Fundamentally, the same lesion as was described in November exists; however, there is new bone production in the mandible which involves approximately three-fifths of the mandible. Only the posterior ramus in the right side is of normal structure. Aside from the blotchy new bone production, there are areas of loss of bony structure, and trabeculae are distorted."

A bone assay performed at Lutheran Hospital in January, 1957, revealed that there were variations in the bony structure of the dorsal spine, along with flattening and deformity of the cervical segments, specifically confined to C-5, C-6, and C-7. There was new bone production anteriorly (Fig. 3).

The patient was admitted to Lutheran Hospital on December 3 for a biopsy. At the same time body fluids were drawn and analyzed, and the serum alkaline phosphatase was found to be elevated, with all other levels remaining within normal limits. The results of the blood chemistry analysis were as follows:

Calcium	10.8 mg.
Phosphorus	3.6 mg.
Alkaline phosphatase	7.2 Bodansky units
Acid phosphatase	0.4 King-Armstrong unit
Albumin-globulin ratio	1.6
Kline	Nonreactive
White blood cells	12,700
Microhematocrit	46 per cent
Urine	Clear, negative for albumin, sugar, and sedimentation

On December 4 the patient was taken to the operating room. Anesthesia was induced with 2.5 per cent Pentothal sodium and surgically carried with endotracheal nitrous oxide-oxygen-ether. Because the greater area of rarefaction was closer to the lower border of the mandible, an external approach was utilized.

Antibiotic therapy was instituted, and the postoperative course was uneventful. The patient left the hospital on the fourth postoperative day. The pathologic report was as follows:

Gross: Dr. Quentin Howard reported that the specimen consisted of thirty-four fragments of bony tissue measuring from 0.1 to 1.0 cm. in greatest dimension. The

bone was grayish white mottled grayish pink with several small blood clots attached. The specimen was embedded in toto.

Microscopic: Multiple decalcified sections showed numerous similar fragments and masses of bone composed of trabeculae which varied greatly in size and shape and which frequently were ragged and showed varying degrees of calcification. Between the trabeculae was fibrous tissue stroma containing many fibroblasts, lymphocytes, plasma cells, and neutrophils. There were zones of osteoblastic and osteoclastic activity, and there were occasional multinucleated giant cells. No malignant tumor was present.

Diagnosis: Dr. William Sinclair, Jr., diagnosed the condition as chronic inflammation of bone (see discussion).

Fig. 3.

Fig. 4.

Fig. 3.—Case 1. Roentgenogram showing flattening and deformity of cervical segments with production of new bone anteriorly.

Fig. 4.—Case 1. Roentgenograms taken one year later show complete involvement of left mandible with spreading to right side.

A final diagnosis of early osteitis deformans was made. The patient was then presented to the Tumor Conference at Lutheran Hospital, where this diagnosis was supported. No particular form of treatment was recommended by the conference, inasmuch as nothing specific is known for the disease.

Subsequent periodic examinations showed a progressive skeletal distortion, spinal bowing, and an increased degree of mandibular trismus. Follow-up radiography in October, 1957, was reported by Dr. Jean A. Groh as follows:

> "Repeat examination of the mandible shows again the rather extensive lesion involving the left mandible and anterior portion now also progressing definitely up on the right side and spreading along the right anterior mandible. The lesion presents the same picture. There is a definite loss of the normal bone pattern and trabeculation. We have a deformity and a widening with a thin cortex and the areas of increased density mingled with areas of radiability. (Fig. 4.)
>
> "As far as the pelvis is concerned, we see a bony spur projecting into the pelvic space opposite the right acetabulum and right ischium.

Fig. 5.—Case 1. Roentgenogram of cervical spine showing lesion progressing to involve C-2 through C-7.

> "In the cervical spine we have now the same change as a progressive lesion which now involves not only C-5, C-6, and C-7, as in the previous examination of last January, but also shows progressive disease along the upper portion of the cervical spine as high as C-2. These bodies now also show spur formation, roughening, irregularities, and considerable new bone production anteriorly with a wide space between the air passages and the spine. Along with that, now we have a very definite kyphosis, the height of the curve being approximately opposite C-5. The lower cervical segments which were previously involved do not show any severe advanced lesion any more than in the last examination, but the lesion is now spreading up along the cervical region anteriorly involving the upper cervical spine." (Fig. 5.)

Case 2.—R. G., a 72-year-old white male laborer, presented himself to our office with a chief complaint of pain in the roof of his mouth, radiating to his upper jaw, and inability to wear his upper denture.

The patient stated that he was in apparent good health until about one year before, at which time he began experiencing difficulty in wearing his upper denture, which he then discarded. His lower prosthesis continued to serve him adequately. Shortly thereafter, he began to notice a vague intermittent ache in his palate and in his left maxilla. This subsided after several months and recently recurred, accompanied by extremity joint pains.

His past history was unreliable and noncontributory. He denied all diseases categorically. A full-mouth extraction had been performed six years previously without apparent complication.

He denied drug, alcohol, or tobacco consumption, and stated that he had not experienced anorexia, insomnia, and weight loss. Family history was vague and unreliable.

Review of the systems was essentially negative, with the exception of his present complaint.

Examination revealed a well-developed elderly white man sitting upright and in no acute distress.

Fig. 6.

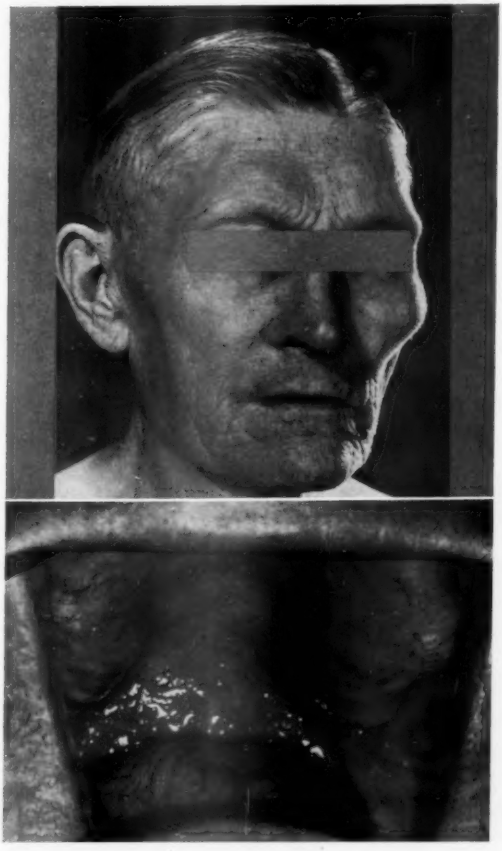

Fig. 7.

Fig. 6.—Case 2. Photograph showing prominent left malar bone.
Fig. 7.—Case 2. Photograph showing enlarged alveolar ridge (left).

His face was slightly asymmetrical (Fig. 6) with the left side showing some enlargement, particularly in the area of the left malar bone. There was no lymphadenopathy, paralysis, tenderness, edema, or pigmentation.

Fig. 8.

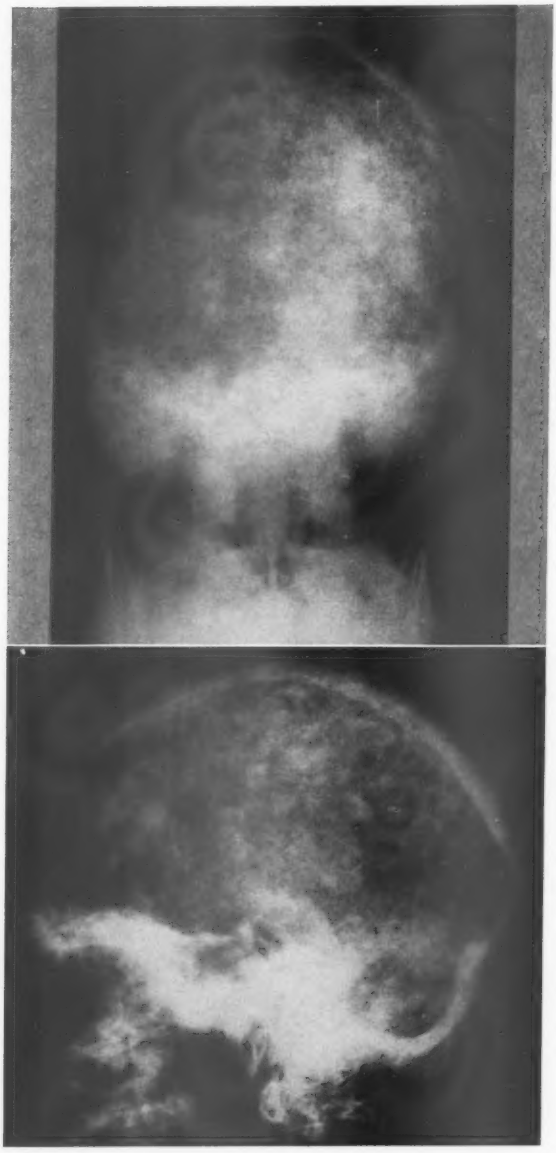

Fig. 9.

Fig. 8.—Case 2. Radiograph showing cotton wool appearance of calvarium.
Fig. 9.—Case 2. Lateral radiograph showing involvement of skull and facial bones.

He was edentulous and was wearing only his lower prosthesis. The upper alveolar ridge was enlarged on the left side; it was firm, smooth, and nontender (Fig. 7). All other structures were essentially normal.

Initial roentgenograms showed a generalized density of the maxilla which was quite profound. There were no rarefying areas, and in the alveolar ridges the density was almost complete. There was partial bony obliteration of the left maxillary sinus. The mandible was normal.

Differential Diagnosis.—At the outset the patient refused biopsy. A general physical examination was essentially negative, but with the stipulation by the examining physician that a total bone x-ray survey be made.

This course was pursued, and the resultant radiographs revealed a marked thickening of the skull, accompanied by a cotton wool appearance of the calvarium, with multiple circular areas of increased density (Fig. 8). There was further evidence of extremely dense, sclerotic bone in the maxilla, left malar bone, pelvis, and lumbar spine (Fig. 9).

A tentative diagnosis of polyostotic osteitis deformans was then made. This diagnosis was confirmed by an extremely high serum alkaline phosphatase fraction in a blood chemistry analysis which yielded the following findings:

Calcium	9.3
Inorganic phosphorus	3.7
Alkaline phosphatase	56.8
Acid phosphatase	0.21
Albumin-globulin ratio	2.0
Kline	Negative
Hematocrit	38 per cent
White blood cells	9,200
Sedimentation rate	2.8 mm.

No treatment was recommended. The patient had subsequently developed some degree of skeletal bowing and a total deafness.

CASE 3.—Although the following does not represent a case of early or undiscovered osteitis deformans, it has been included because it manifests an interesting problem in the differential diagnosis of the disease.

C. H., a 60-year-old unemployed white man with late Paget's disease, was referred to our office with the chief complaint of pain and swelling in the lower left facial area (Fig. 10).

He stated that the onset of his present illness began five weeks previously, at which time six teeth were extracted from the lower left quadrant without apparent complication. On the fifth postoperative day he developed swelling and tenderness over the left side of his lower jaw, which increased until the ninth day, when spontaneous drainage of purulent material from the molar extraction site occurred. The swelling regressed, and the patient consulted his physician, who administered penicillin and advised saline rinses.

Two weeks later swelling and pain recurred in the same area, and the patient consulted his dentist, who removed several pieces of sequestered bone from the area, obtained additional purulent drainage, and inserted an iodoform gauze drain. When the swelling did not regress after several days, dental radiographs were taken which showed evidence of further sequestration (Fig. 11). A temperature reading of 101° F. was noted, and the patient was referred to our office with the tentative diagnosis of osteomyelitis.

In relating his past history, the patient stated that he had had Paget's disease involving his skull, spine, and legs for twenty-two years. He had not had any jaw involvement. He denied all other diseases prior to the time of this occurrence and all illnesses similar to his present complaint.

He denied drug, alchohol, or tobacco consumption and stated that he had been unable to obtain employment for about fifteen years because of his deformity.

Fig. 10.—Case 3. Photograph showing swelling in left facial area.

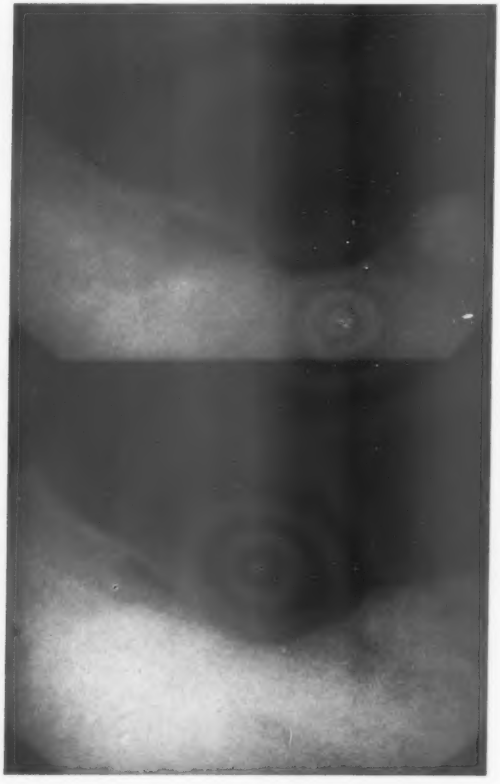

Fig. 11.—Case 3. Radiograph showing region of sequestrum formation.

His family history was noncontributory. A review of the systems indicated a great deal of disability due to his disease but was essentially negative with respect to the head, mouth, and jaws, with the exceptions of his enlarged calvarium and his present illness.

Examination revealed a deformed, grotesque man in no acute distress. His head was abnormally large, bent down, and almost immobile, and facies were distorted and asymmetrical. Spinal scoliosis and bowing of the skeletal frame were evident (Fig. 12). Mottled skin pigmentation was present, and there was an indurated swelling over the left mandibular angle extending forward to the region of the second premolar. No lymphadenopathy or paresthesia was present.

Fig. 12.—Case 3. Photograph showing bowing of skeletal frame.

Intraorally the ridges appeared slightly enlarged but within normal limits, and a gauze drain was inserted into the alveolus in the region of the lower left first molar (Fig. 13). The area was tender, and yellowish purulent material could be expressed around the drain. Temperature was 101° F.

Fig. 13.—Case 3. Photograph showing enlargement of alveolar ridge.

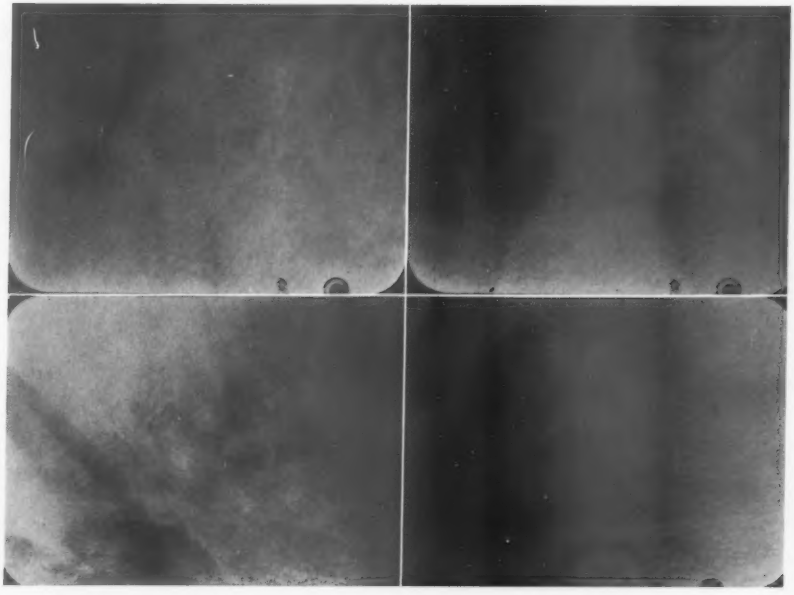

Fig. 14.—Case 3. Radiographs showing loss of trabecular architecture.

Adequate extraoral radiographs could not be taken because of the patient's inability to elevate his head. Dental films revealed spotty loss of density and several areas of circumscribed bony sequestration in the left molar area.

After treatment with antibiotic drugs was instituted, an intraoral operation for removal of bone sequestra was performed. An incision over the posterior alveolar ridge permitted exploration and curettement of the area. Three pieces of sequestered bone of rubbery consistency, together with pieces of soft granulation tissue, were removed and sent to the pathologist for examination.

The pathologist's report stated that the bone showed changes consistent with osteomyelitis. Specimens were necrotic and lacked cellular elements. Margins removed from the intact bone, however, showed fibrous tissue replacement of marrow spaces and osteoblastic-osteoclastic activity. The possibility of the infection having been superimposed on pagetoid mandibular bone was not excluded.

The patient's swelling and pain subsided, and no further sequestration occurred. A total bone survey and blood chemistry were then performed.

The bone survey noted that the spine, pelvis, skull, and chest showed typical bony changes of polyostotic osteitics deformans with generalized and spotty osteoporosis, irregular areas of chalky increase in density, and increased and coarsened trabeculation. Dental films showed osteosclerosis with irregularly dense areas of radiolucency and radiopacity in the mandible and maxilla with loss of trabecular architecture due to Paget's disease (Fig. 14).

The blood chemistry assay was within normal limits with the exception of the alkaline phosphatase level, which was 41.5 Bodansky units.

A final diagnosis of acute osteomyelitis superimposed upon osteitis deformans of the mandible was then made.

One year later the patient developed a fibrosarcoma of the right femur, and a mid-thigh amputation was performed. He died a week later of acute pulmonary infection.

DISCUSSION

The three cases presented are of particular interest from a dental standpoint because of their clinical similarity to other bony lesions of the jaws. Although pain is inconstant and seldom severe, it may resemble many of the neuralgias that we find about the jaws. Only after a complete history, roentgenographic examination, and blood chemistry analysis can we be certain of the type of jaw lesion that we have encountered.

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PAGET'S DISEASE WITH OSTEOGENIC SARCOMA OF MAXILLA

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THE oral surgeon is occasionally called upon to render a diagnosis of some facial bone disorder of systemic origin which may be discovered during routine intraoral clinical or roentgenographic examination. Any unusual appearance of bone in the dental film should be closely studied for correct interpretation.

Paget's disease, osteitis deformans, an osteodystrophy of unknown etiology, is such a disorder and may show oral changes before other symptoms are manifest. It is characterized by simultaneous resorption and excessive formation of bone in one (monostotic) or more (polyostotic) parts of the skeleton. The bones most frequently affected are the skull, tibia, femur, pelvis, and spine. The marked tendency toward new bone formation and destruction is reflected in the high levels of serum alkaline phosphatase and frequently results in enlargement and deformity of the affected bones. Excessive changes of the head and the face may give the victim an apelike appearance.

Histologically, the disease usually shows replacement of marrow spaces by connective tissue and notable osteoblastic and osteoclastic activity with the formation of numerous small irregular spicules of bone. The irregular apposition and resorption lines present a "mosaic" pattern (Fig. 3).

In spite of the increased turnover of bone, the calcium and phosphorus are usually within normal limits. The alkaline phosphatase is generally elevated—markedly so in the active stages.

X-ray examination usually shows rarefaction and increased density of the bone. The head often enlarges, and the outer table of the skull may present a fuzzy appearance. In the oral cavity, the most extensive changes are usually found in the maxilla. Hyperostosis is produced by large deposits of porous bone evidenced by a widening and an increase in the size of the trabeculae, with areas of increased density. These radiolucent areas produce the characteristic "cotton wool" appearance of the bone. In the early stages, there often is an absence of the lamina dura around the roots of the teeth. In the advanced stages, sclerotic bone, hypercementosis of the roots, and more than normal pulp calcifications are found. It has been noted that the jaw picture may antedate by several years the radiographic appearance of the disease in the rest of the skeleton.

There is a definite relationship between Paget's disease and osteogenic sarcoma. Some investigators claim that it may be as high as 40 per cent. The chief characteristic is a malignant change of the bone-producing cells, although

bone formation and bone destruction may go on in the same tumor. Changes which may be found are bone destruction, periosteal reactions, and soft tissue changes. These may be manifested by the moth-eaten appearance of the surrounding bone, the radial spicules of bone giving it a "sun ray" effect, or a periosteal reaction which may be noted in adjacent areas.

Osteogenic sarcoma arising in Paget's disease gives evidence of the malignant change by the rapid appearance of a new mass. The lesion may show both bone destruction and new bone laid down. Its treatment is very unsatisfactory. Surgery, radiation, or a combination of both have been used rather extensively. The prognosis is poor, particularly in patients beyond the age of 50 years. The case with a long history and a well-differentiated and localized tumor made up of adult tissue has a greater degree of hope than the one whose history shows it to be rapidly growing and whose histology denotes a very undifferentiated osteolytic tumor.

The following case of a sarcoma developing in bone involved by Paget's disease occurred in a patient who had been under observation for several years —first for an arthritis of the hip and later for Paget's disease. The hip, pelvis, and maxilla were involved. It was interesting that changes in both the jaw and the hip were noted by both the medical and the dental services at the same time. Such changes can bring the patient to the oral surgeon, thus posing a problem in oral or radiographic diagnosis.

CASE REPORT

Chief Complaint.—A 57-year-old woman was admitted to Worcester City Hospital on Oct. 14, 1954, with severe pain in the right groin and leg.

History of Present Illness.—The patient had suffered a steady pain of uniform intensity in the right groin, posterior thigh, and calf for the past two years. The pain was aggravated by ambulation and, in the main, was not relieved by analgesics and sedatives which she received periodically from the outpatient department of the hospital. As a result, she led a bed-to-chair existence during this time. About one year prior to admission, x-ray examination of the right pelvis disclosed Paget's disease (Fig. 1).

Twenty-four hours before admission, the patient suddenly felt an excruciating and sharp pain in the right thigh. The pain was so severe that she called her physician, who referred her to the hospital. On admission, a dental consultation was requested by the medical service.

Past History.—The patient had a history of six previous hospital admissions for her pregnancies. She had a hemorrhoidectomy in 1944, but no previous serious illness. Menopause started at the age of 39. Her appetite had been fair and her bowels regular. She had no respiratory or genitourinary disorders, but during the past two years she had lost about fifteen pounds in weight. She had no known allergies, and neither smoked nor indulged in alcoholic beverages.

For the past year she had been treated for a hip disturbance at the arthritis clinic of the hospital's outpatient department. She stated that the pain had been steadily getting worse and complained that the right hip frequently "snapped" out of place. Records showed no gross shortening of the right leg, although there was some limitation of hip movement.

On July 7, 1953, all the upper teeth were extracted by the dental service of the outpatient department. Intraoral roentgenograms showed typical changes. The roots of several teeth showed resorption and absence of lamina dura, and the maxilla disclosed several areas of osteoporosis and increased densities (Fig. 2). A biopsy of a section of the alveolar process was diagnosed as Paget's disease (Fig. 3).

Fig. 1.—Paget's disease with sclerosis and osteoporosis of the right ilium.

Fig. 2.—Patchy osteoporosis with root resorption and loss of lamina dura.

Physical Examination.—Examination revealed an agitated, obese woman in traction, lying in bed with rapid, grunting respirations and complaining of severe pain of the right leg and hip. The chest was clear to percussion and auscultation, although there were excessive respirations. There was some cardiac enlargement, and a grade III apical, systolic, soft-blowing murmur was audible. There was a hard, irregular, nontender, immobile mass in the right lower quadrant. The patient had a large symmetrical head, with facies more prominent than normal; she was very stoop shouldered, and her general appearance was apelike. Other findings were not significant.

Her temperature was 98.6° F.; the pulse, 72; and the respirations, 40. The blood pressure was 140 systolic and 80 diastolic.

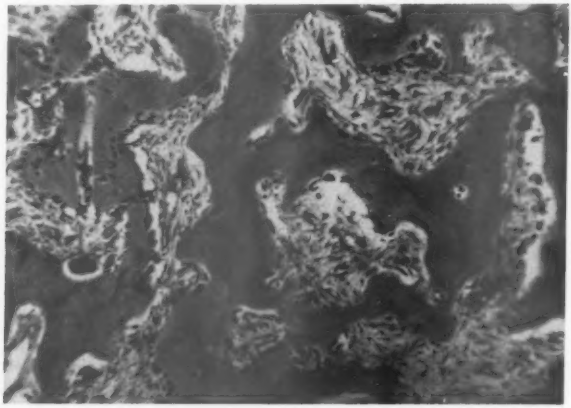

Fig. 3.—Paget's disease of alveolar bone with a typical mosaic pattern showing replacement of the marrow by connective tissue.

Fig. 4.—Diffuse mass of the left maxillary alveolar process with a pronounced bulge of the upper lip.

Oral Examination.—The patient was edentulous; the gingivae were pale, and the tongue was dry and coated. The palate was broad, and the alveolar process was thick and prominent. The bulkiness of the alveolar ridge caused a marked protrusion of the upper lip. The left tuberosity was about one inch in width and contacted the edentulous left molar region of the mandible. She was unable to close and occlude her jaws properly because of an impingement of the left ramus. The mandible was normal in size. There was a pronounced, fixed, painful, nonfluctuant, rapidly growing mass in the upper left anterior maxilla (Fig. 4).

Laboratory Examination.—Urinalysis was negative. Examination of the blood showed a red cell count of 3,200,000 with a hemoglobin of 10.5 Gm. per 100 c.c. The white cell count was 8,650 with 68 per cent neutrophils. The serum calcium was 9.6 mg., the serum phosphorus was 4.1 mg. per 100 c.c., and the alkaline phosphatase was 14.7 Bodansky units. The Hinton test was negative.

Roentgenographic Examination.—Roentgenograms taken on June 23, 1953, disclosed Paget's disease of the right ilium (Fig. 1). Roentgenograms of the maxilla revealed the

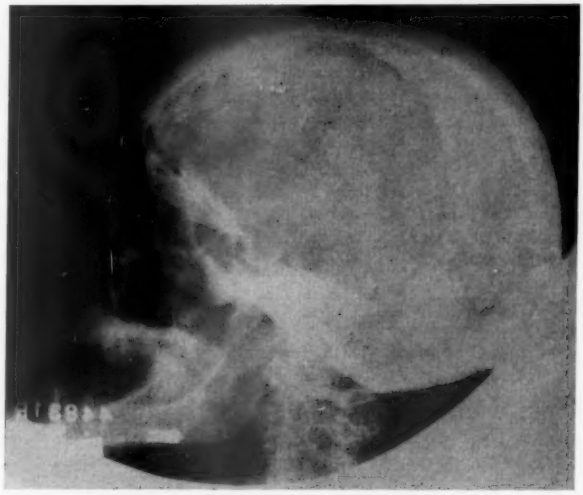

Fig. 5.—Osteoporosis circumscripta cranii involving the parietal and occipital bones. Note cotton wool appearance of the maxilla.

Fig. 6.—Large radiopaque mass of the pelvis indicating sarcomatous degeneration of Paget's disease.

typical spongy, mottled, and cotton wool appearance of combined rarefaction and ossification (Fig. 2). Roentgenograms of the skull showed a normal appearance of the outer plate with large irregular areas of diminishing bone intensity involving the left parietal and occipital bones (Fig. 5). Roentgenograms taken on Oct. 28, 1954, disclosed Paget's disease, osteitis deformans, of the right ilium plus a large structureless mass extending into the pelvis which was diagnosed as a possible sarcomatous degeneration of Paget's disease (Fig. 6).

Provisional Diagnosis.—(1) Pathologic fracture of right ilium; (2) Paget's disease with sarcomatous degeneration; (3) osteogenic sarcoma of the maxilla.

Hospital Course and Treatment.—The patient was placed on a regimen of penicillin together with analgesics and sedatives for the relief of pain. These included aspirin, codeine, Demerol, paraldehyde, Thorazine, and placebos. She was confined to bed and placed in traction. Supportive treatment was instituted. She was given 2,500 c.c. of whole blood, but her hemoglobin remained low during her stay in the hospital.

On Oct. 29, 1954, a biopsy of her hip disclosed the lesion to be an osteogenic sarcoma. She had difficulty in eating because of pain and interference with the left ramus. In addition, the anterior maxillary alveolar ridge was beginning to increase in size, causing her additional pain and discomfort. A provisional diagnosis of osteogenic sarcoma of the maxilla was made, and it was decided to perform a biopsy of the mass and to revise the entire alveolar ridge in order to improve it.

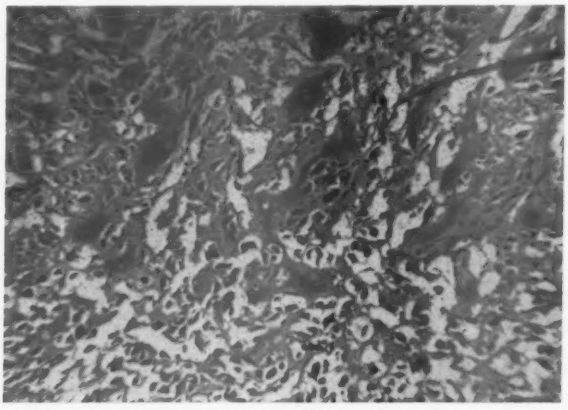

Fig. 7.—Photomicrograph of osteogenic sarcoma arising from area of Paget's disease. Note large number of abnormal osteoblastic cells and formation of osteoid tissue.

On Nov. 16, 1954, the patient was premedicated with 75 mg. of Demerol and $\frac{1}{150}$ gr. of atropine sulfate one hour before operation. The face and mouth were then prepared and draped in the usual manner. A 2 per cent solution of Xylocaine with 1:100,000 epinephrine was injected locally, by means of tuberosity and infraorbital injections. Injections were also made into the anterior and posterior palatine foramina. An incision was made along the entire crest of the maxillary alveolar ridge from the left tuberosity to the right tuberosity and carried through to the periosteum. The mucoperiosteum was reflected on the palatal and buccal aspects with periosteal elevators. This exposed the entire alveolar ridge. The mass was soft, having the consistency of fibrous tissue, and was easily excised with the scalpel. Both tuberosities were reduced, and the entire ridge was contoured and revised by removing the excessive osteoporotic bone with rongeurs. All undercuts were relieved and sharp edges were trimmed and smoothed with bone files. The loose spicules of bone were removed, and the excess of the flap was trimmed and approximated with interrupted silk sutures. The returned biopsy report indicated osteogenic sarcoma (Fig. 7) arising from an area of Paget's disease.

During the operative procedure profuse bleeding was encountered. This was controlled by epinephrine-soaked packs and by suturing of the mucoperiosteal flaps in close apposition. This procedure also eliminated the interference with the patient's jaw movements.

From Dec. 4 to 17, 1954, external pelvic roentgen therapy was applied, and the patient received the total palliative dose of 2,200 roentgens to the tumor at the rate of 200 r a day. Following this, there was some improvement and she was able to sit up in a chair. The radiologist declined to irradiate the maxilla. After several weeks, it was observed that the sarcoma of the maxilla began to recur and cause her pain (Fig. 8). She was very anxious to go home and was discharged from the hospital on Dec. 24, 1954, with prescriptions for sedation.

Fig. 8.—Recurrence of osteogenic sarcoma following alveoplasty of maxilla.

Second Admission.—The patient was readmitted on Jan. 5, 1955, with intractable pain. She complained that she had not been able to refill her prescription for pain. She was unable to eat and had become a nursing problem. She was again given supportive treatment.

Shortly after admission, a consultation with the x-ray department regarding further radiotherapy was requested. It was decided that such a course was useless, and no further therapy was given.

A chordotomy was proposed by the neurological service, but the patient refused to undergo the procedure. She was discharged on Feb. 4, 1955, to the outpatient department for supportive care. The patient died at home on Feb. 19, 1955. No post mortem was performed.

SUMMARY

Paget's disease reaches its greatest height of destructive activity in later life. The onset is insidious, and often patients are unaware of any disorder until routine roentgenograms reveal the presence of hyperostosis or the bones of the face and jaws become noticeably altered.

Cystic spaces are frequently seen, due to liquefaction of the connective tissue and bone, and profuse hemorrhage is often encountered during surgery.

Persons afflicted with Paget's disease have been found to have a high incidence of osteogenic sarcoma. Some microscopic sections of bone may present a benign Paget's lesion and also show sarcomatous degeneration arising in the same area.

The therapeutic results of osteogenic sarcoma in general are poor. Radiotherapeutic methods will have to be improved if satisfactory control of the disease is to be achieved. In the aged and feeble patient with an advanced lesion, palliation is all that can be offered.

The oral surgeon should be familiar with the diagnosis and treatment of such disorders, especially those involving the face and jaws, and he should see to it that the patient receives proper treatment. It cannot be overemphasized that early recognition and prompt institution of treatment are vital.

The author wishes to acknowledge his thanks to Dr. Gerald Shklar of the Department of Oral Pathology, Tufts University School of Dental Medicine, for his assistance.

60 Franklin St.

A GIANT-CELL TUMOR OF THE MAXILLA IN AN AREA OF OSTEITIS DEFORMANS (PAGET'S DISEASE OF BONE)

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THE occurrence of a giant-cell tumor of bone in an area of Paget's disease is an extremely rare finding and has not been reported previously in the jaws. Jaffe[3] has reported a case of a giant-cell tumor arising in a skull involved by osteitis deformans. We have recently had the opportunity to study a case of central giant-cell tumor of the maxilla apparently arising in bone involved by Paget's disease. The latter condition was not clinically obvious in this 66-year-old female patient and first came to light as a result of microscopic studies of tissue removed from a multilocular radiolucent area of the maxilla. Follow-up radiographic surveys and blood studies confirmed the diagnosis of Paget's disease of bone. The original radiolucent lesion was diagnosed as giant-cell tumor and presented the microscopic features of giant-cell tumors found elsewhere in the skeletal system.

CASE HISTORY

Chief Complaint.—H. F., a 66-year-old married white woman, was first seen on April 6, 1954, because of a recurrent bitter taste and a painless enlargement of the anterior maxilla.

History of Present Illness.—The patient had a full maxillary denture for many years, and because of this prominence in the anterior area she was no longer able to wear it comfortably.

Past Medical History.—The patient had a right mastectomy in 1938 because of cystic mastitis. She had been treated for many years for an enlarged heart and recurrent bouts of palpitations of her heart. The remainder of her history and her family history were essentially negative.

Examination.—Clinical examination revealed a 3 by 2 cm. enlargement of the midline of the maxilla which virtually obliterated the mucobuccal fold. The maxilla was edentulous, but the patient still retained her anterior mandibular teeth.

General physical examination demonstrated remarkably little pathology. There was some prominence of the frontal bone, but otherwise the patient had no deformity that could be associated with osteitis deformans. She had grade II arteriosclerotic changes in the fundi of her eyes. The remainder of her physical examination was essentially negative.

Intraoral dental films on April 6, 1954, demonstrated the presence of multiple radiolucent cystic-like lesions in the anterior maxilla (Fig. 1); the largest of these areas measured 1.1 by

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1.4 cm. and was present to the right of the midline. Several additional cystic-like areas, measuring an average of 0.4 cm., were present in the midline and right anterior maxilla. The surrounding alveolar bone presented a pattern of extremely fine trabeculation.

Interpretation: Multiple cystic lesions of the anterior maxilla.

Treatment and Course.—On April 9, 1954, under local anesthesia and premedication of atropine, 1/150 gr., and Nembutal, 0.75 gr., the area was exposed by reflection of a flap of the buccal mucoperiosteum of the maxilla. The cortical bone to the left of the midline was found to be eroded, and a brownish mass was readily revealed beneath it. With hand chisels, the cortical bone was removed to reveal a tumor mass present on both sides of the midline and measuring about 2.5 by 2 cm. The mass was completely enucleated, and several septa were

Fig. 1.—Intraoral radiograph of the maxillary central incisor area. Note the extremely fine trabeculation of the alveolar bone and the multilocular area of radiolucency consisting of one large cystlike area and several smaller areas of radiolucency.

Fig. 2.—Lateral projection radiograph of the skull. Note the marked thickness of the calvarium and the mottled opacity throughout the skull, presenting the typical "cotton wool" appearance of Paget's disease of bone.

found dividing the cavity within the bone. The entire area was treated with phenol and alcohol application. The tissue was then returned to position and sutured with 000 black silk sutures. The patient had a good postoperative course, and on April 17, 1954, the sutures were removed.

Laboratory Findings.—The following laboratory findings were reported:

Biopsy report (April 13, 1954): Central giant-cell tumor of bone with osseous changes suggestive of Paget's disease.

Blood studies (April 20, 1954): Hemoglobin and complete blood count were normal. Chemistry was as follows: calcium, 9.9 mEq./L.; phosphorus, 3.1 mEq./L.; alkaline phosphatase, 28 Bodansky units.

Urinalysis: Normal findings.

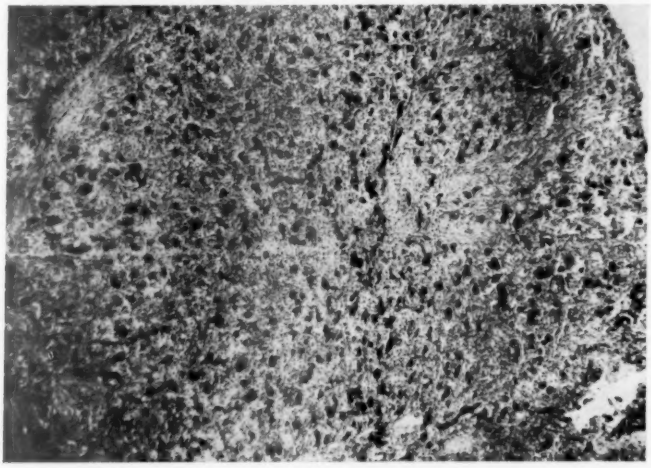

Fig. 3.—Photomicrograph of the central portion of the tissue removed from the area of radiolucency (low power). Note the large number of giant cells scattered homogeneously throughout the tissue. (Hematoxylin and eosin stain. Magnification, ×80; reduced ⅝.)

Roentgenographic Studies (April 24, 1954).*—"Examination of the skull by lateral projection reveals the classical appearance of well-advanced Paget's disease with very marked increase in calvarial thickness (Fig. 2). There are no remaining upper teeth or root fragments. The lower molars are absent.

"Frontal film of the thorax shows cardiac enlargement, with a transverse diameter of 16.5 cm. There is relative prominence of the left ventricle. The aorta is not enlarged but does contain calcium in the arch. There is no indication of active pulmonary disease.

"Survey of the low back and pelvis reveals additional very extensive skeletal alteration from Paget's disease, involving all of the right innominate bone and the sacrum. Paget's disease of the first, third, and fifth lumbar vertebrae and of the eleventh and twelfth dorsal vertebrae is evident.

"*Interpretation:*

1. Unusually advanced Paget's disease of the skull, several vertebrae, sacrum, and right innominate bone.
2. Cardiac enlargement and arteriosclerosis.
3. Osteoarthritis of the spine.
4. Left lumbar list with compensatory curve."

*John Turner, M.D., Radiologist, Wesson Memorial Hospital, Springfield, Massachusetts.

Fig. 4.

Fig. 5.

Fig. 4.—Photomicrograph of the peripheral area of the lesion. Note the numerous giant cells within the tissue at the right. The giant cells are scattered throughout the tissue. Note the small irregular spicules of bone at the left of the field, with replacement of the marrow spaces by connective tissue. (Hematoxylin and eosin stain. Magnification, ×80; reduced ⅝.)

Fig. 5.—High-power magnification of section shown in Fig. 4. Note the evenly spaced multinucleated giant cells lying in a matrix of spindle-shaped or polyhedral cells. Some inflammatory cells are present. Some of the giant cells contain upward of fifty nuclei. (Magnification, ×310; reduced ⅝.)

Fig. 7.

Fig. 6.

Fig. 6.—Medium-power magnification of the bone tissue shown in Fig. 4. Note the replacement of the marrow spaces by fibrous connective tissue and the numerous irregularly shaped spicules of bone. There are numerous multinucleated osteoclasts lying in lacunar spaces, and there is evidence of layers of osteoblasts laying down osteoid material. (Magnification, ×220; reduced ⅝.)

Fig. 7.—High-power study of tissue shown in Fig. 6. Note the multinucleated osteoclasts and the osteoblasts laying down the light-staining osteoid material. Note the irregular scalloped margin of the bone and the numerous curvilinear, darkly staining lines through the bone spicules, presenting the "mosaic" pattern characteristic of Paget's disease of bone. (Magnification, ×310; reduced ⅝.)

The patient was last seen in January, 1956, with no further evidence of recurrent pathology of the maxilla. Her family physician is presently attending her in reference to the observation of Paget's disease.

The patient was returned to her dentist for follow-up and was not seen again until Dec. 14, 1954; intraoral films taken at that time showed obliteration of the cystic-like area. She has had no further complaints in this area, and there has been no recurrence of the tumor-like growth. Follow-up x-ray films taken on April 1, 1955, revealed a pattern similar to that seen in the previous film. All bones of the calvaria were found to be involved with alteration of the cranial foramina.

Microscopic Studies.—Following enucleation of the tissue mass from the multilocular area of radiolucency in the maxilla, the tissue was studied microscopically. The sections revealed a large mass of tissue with numerous bony spicules at the periphery of the soft tissue. The tissue was found to be composed of large numbers of multinucleated giant cells lying in the cellular stroma of spindle-shaped or ovoid cells. The stromal network presented numerous small blood vessels. The giant cells were evenly distributed throughout the tissue fields (Fig. 1) and were found to contain large numbers of nuclei (Fig. 5). The giant cells stained heavily with hematoxylin and eosin and in some instances presented a granularity of the cytoplasm (Fig. 5). The cells of the stromal network were regular in size, shape, and chromaticity and varied only from spindle-shaped to ovoid. There was no evidence of mitotic activity. A diagnosis was made of giant-cell tumor of bone, benign. The lesion was also found to be encapsulated with a band of dense fibrous connective tissue.

The bone at the periphery of the lesion presented an unusual appearance which at first was thought to be a reactive alteration to the expanding giant-cell tumor. On closer inspection, the bone revealed all the characteristic changes of Paget's disease. The bone was composed of numerous irregularly shaped spicules and the marrow spaces had been replaced by dense fibrous connective tissue (Fig. 4). There was evidence of active osteoclastic resorption with numerous multinucleated osteoclasts lying in lacunar spaces. The bone spicules presented an irregularly scalloped margin (Figs. 6 and 7). In addition to the osteoclastic activity, there was active osteogenesis, as evidenced by numerous osteoblastic cells laying down osteoid (Figs. 6 and 7). The bone spicules themselves showed numerous darkly staining lines characteristic of the "mosaic" pattern. The appearance of the bone strongly suggested the possibility of Paget's disease. Radiographic and blood studies confirmed the diagnosis.

DISCUSSION

The occurrence of a giant-cell tumor in an area of osteitis deformans is extremely rare and has not been reported previously in the jaws. No attempt will be made in this report to suggest the nature of these lesions. The diagnosis of Paget's disease was made on the basis of the microscopic findings as well as the blood studies and radiographic survey. The blood studies revealed a markedly elevated alkaline phosphatase (28 Bodansky units). Roentgenologic studies revealed typical alterations characteristic of Paget's disease. There was extensive involvement of the skull, right innominate bone, sacrum, and numerous vertebrae. The microscopic changes were at first thought to be an unusual bone reaction to the expanding giant-cell tumor. The laboratory studies substantiated the suspicion of possible Paget's disease.

The central neoplastic lesion was diagnosed as giant-cell tumor and was felt to be comparable to lesions diagnosed elsewhere in the body as giant-cell tumor. There has been a tendency in recent years to call giant-cell lesions of the jaw *giant-cell reparative granulomas.*[3] Jaffe describes giant-cell reparative granulomas of the jaws as occurring primarily between the ages of 10 and 25, being

found more commonly in the mandible, and being more common in females. The giant cells are sparse, small, and unevenly distributed.[3] There is a loose vascular stroma, and the giant cells are often clumped in areas of hemorrhage.[3] The true giant-cell tumor is described as occurring between the ages of 25 and 40 and consisting of large numbers of giant cells distributed evenly throughout the tissue fields. The giant cells are found in a stroma of spindle-shaped or polyhedral cells.[3, 4] Jaffe considers true giant-cell tumors of the jawbones to be very rare and claims that he has seen only one case.[3] Other authors do not feel that giant-cell tumor of the jaws is a rare lesion. Lichtenstein[7] lists the sites of giant-cell tumor as lower end of femur, upper end of tibia, lower end of radius, jawbones, upper end of humerus, upper femur, upper end of fibula, and patella. From the microscopic appearance of the tissue, our case would meet Jaffe's criteria for giant-cell tumor rather than reparative granuloma.

The giant-cell tumor described is interpreted as a benign lesion. Many giant-cell tumors have been reported as becoming malignant,[8, 9] however, and this possibility should be mentioned.

Russell[12] has recently reported three cases of malignant giant-cell tumors arising in foci of Paget's disease. Jaffe and his colleagues[4] graded giant-cell tumors as follows: Grade I—least aggressive, stroma with no atypism; Grade II—compact cellular stroma with some evidence of atypism; Grade III—sarcomatous type of stroma. Grade III lesions are malignant and metastasize. Willis[17] felt that some giant-cell tumors not initially distinguishable from benign lesions are later found to invade and metastasize. Murphy and Ackermann[8] note that 10 per cent of all giant-cell tumors are malignant at the time of first examination and others may become malignant. They discuss two cases of benign lesions becoming transformed into fibrosarcomas.

While giant-cell tumors of bone are not commonly found in cases of Paget's disease, malignant tumors of bone (notably osteogenic sarcoma) frequently develop in this interesting disturbance of bone. In fact, three of the five patients originally described by James Paget[10] developed osseous "cancerous growths." Since Paget's disease is often complicated by malignant bone tumors, our case will be carefully followed despite the interpretation of a Grade I benign giant-cell tumor.

Pain was not a feature of the lesion in this patient, although pain has been noted as a common finding in Paget's disease.[5]

SUMMARY

A case of giant-cell tumor of the maxilla arising in a focus of Paget's disease has been presented. The criteria for the diagnosis of giant-cell tumor have been discussed.

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MANDIBULAR OSTEOMYELITIS IN DOGS FOLLOWING IRRADIATION

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IN THE treatment of cancer of the head and neck, it is often necessary to subject the mandible to intensive courses of irradiation. Osteomyelitis of the mandible following such irradiation was reported in France as early as 1922 by Regaud.[1] He observed that initially there was no line of demarcation between the necrotic and viable bone. After the infection was established, however, there was an involucrum at the border of the irradiated field, followed by sequestration and healing.

This complication of irradiation was first reported in the United States in 1924 by Blum.[2] It was then termed "radium jaw," and it resulted from the commercial use of radioactive material which had been accidentally brought into contact with the oral cavity. In 1925, Hoffman[3] reported a case of osteomyelitis following the therapeutic use of radium. In 1926, Lenz[4] recognized that due to the thinness of the overlying mucosa the heavily irradiated mandible was vulnerable to infection. He also recognized the significance of trauma and cited the extraction of teeth as an example. In the same year, Ewing[5] commented on the difficulty of disinfecting such necrotic bone; he stated that it was practically impossible, since the bacteria were safe from leukocytic attack. Ewing also commented on the slowness with which such bone sequestrated.

It has been reported that a dose of 5,000 roentgens or more results in complete devitalization of adult bone.[6] Such necrotic bone will continue to function mechanically for years, however, in the absence of trauma and infection.[7-9]

Bone partially devitalized by irradiation and subsequently fractured as a result of trauma may heal without complications if infection is prevented. This has been observed in numerous fractures of the neck of the femur following irradiation of the pelvis.[10-14] In the hip much soft tissue overlies the bone, and the possibility of compound fracture and infection resulting from irradiation

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is not great. In the oral cavity, however, the mandible is covered by only a thin mucosa, and the possibility of a compound fracture is infinitely greater. For this reason, the incidence of osteomyelitis in mandibular fractures following irradiation is exceedingly high.

Fractures may occur also as a result of infection. Perforation of the mucosa, whether it be from the extraction of a tooth, expulsion of a foreign body, or laceration from trauma, may allow the introduction of microorganisms into the underlying bone. The subsequent inflammatory reaction may result in resorption of bone, thereby producing a fracture.

If a bone lies within the field of irradiation and is subjected to a dose of radiation which will devitalize or partially devitalize it, every effort should be made to avoid subsequent trauma and infection.

Osteomyelitis of the mandible following irradiation has been reported in patients who had been edentulous for many years before receiving roentgen ray therapy.[9, 15-21] Nevertheless, the presence of teeth may contribute to the development of osteomyelitis. Osteomyelitis has occurred in areas where teeth had been extracted shortly before a course of irradiation.[15, 18, 21-23] In addition, there have been reported many cases of osteomyelitis developing where teeth were removed following irradiation.[3, 16, 18, 22, 24, 27, 44] Finally, osteomyelitis has occurred in mandibles in which teeth were present at the time of irradiation and which were not removed until the condition was far advanced, or when the teeth were allowed to exfoliate.[3, 22, 23, 28, 29, 41]

These clinical reports verify the fact that mandibular osteomyelitis following irradiation has developed in a certain percentage of cases under all of the following circumstances: (1) where teeth were removed long before irradiation, (2) where teeth were removed shortly before irradiation, (3) where teeth were removed following irradiation, and (4) where teeth were allowed to remain undisturbed. The variety of circumstances in which osteomyelitis occurs raises the question of whether the exact time of removal of teeth is a critical factor in the development of mandibular osteomyelitis following irradiation.

The reports in the literature indicate that any factor which tends to preserve the integrity of the soft tissue overlying an irradiated mandible may also reduce the incidence of osteomyelitis.

Almost all investigators agree that at least the teeth that are in the field of irradiation should be removed before irradiation is begun.[7, 16, 18, 20-40, 44] Some authors, however, consider preirradiation extraction unnecessary.[8, 15, 42, 43]

Wildermuth and Cantril[15] reported a series in which six of twenty-five patients who had teeth removed before irradiation developed mandibular osteomyelitis following irradiation. Osteomyelitis did not occur, however, in five patients in whom it was necessary to remove carious teeth subsequent to irradiation. They concluded from this study that if modern antibiotics and meticulous care are used teeth can be extracted if they become painful and carious following irradiation, and the incidence of osteomyelitis can thereby be reduced.

The divergent opinions of these and other investigators seem to be based largely on clinical impressions obtained from limited series of personal cases. A review of the literature failed to reveal any reports of controlled experiments performed to determine the optimal time for removal of teeth that lie within the field of irradiation.

In order to study this problem, the following experiment was designed.

METHOD AND GENERAL CONSIDERATIONS

Two groups of adult mongrel dogs were used. Group I was composed of seven dogs in which the mandibles were irradiated with all of the teeth present. This group of dogs was studied to determine whether osteomyelitis would occur following irradiation of the mandibles and, if so, the relationship between the incidence of osteomyelitis and the quantity of radiation delivered. Group II consisted of fourteen dogs in which the right posterior teeth had been removed before the radiation was delivered to the mandibles. This group was studied to determine whether the reactions observed in the dogs of Group I would be modified by the removal of teeth shortly before irradiation.

The animals were housed in the experimental surgery kennels at the University of California Medical Center. They were kept in individual cages and were allowed to exercise in the yard for a period of three hours each morning five days per week.

They were fed a diet of cooked ground horse meat, rolled oats, fish oil, tomato juice, and salt once daily five days per week. On week ends the animals were fed a commerical dry grain preparation reconstituted with water. Both of the diets were of a soft, mushy consistency that did not require chewing. The amount of mastication done by the animals in taking the diet was very slight.

If one of the dogs refused the diet, he was fed raw ground horse meat. This diet also required little mastication but was of a coarser consistency than the regular diet. On the days when an anesthetic was given, the animals received 30 ounces of reconstituted evaporated milk only.

The animals were anesthetized for all surgical, radiologic, and photographic procedures with a solution of 6 per cent pentobarbital sodium, administered intravenously, in the amount of 0.5 c.c. per kilogram of body weight.

The animals were examined at least once a week during the acute phase of the soft tissue reactions. Color photographs were made to record the visual tissue changes at varying intervals. Photographs also were made of the specimens removed at autopsy.

Radiographs were made of each mandible before irradiation and at approximately monthly intervals thereafter. Radiographs were also made of the specimens removed at autopsy.

Following autopsy, the mandibles were fixed in a 10 per cent solution of formalin. Each side of the mandible was then divided into three sections with a band saw. Section A was a cross section through the nonirradiated anterior portion. Section C was a cross section of the irradiated portion. Section B

was a longitudinal section made by dividing the mandible in a sagittal plane extending from Section A to Section C, designed to show the transition from nonirradiated to irradiated tissue. The sections were decalcified, embedded in paraffin, and prepared for histopathologic study. The tissue was stained with hematoxylin and eosin.

Surgical Procedure.—The surgical procedure was standardized so that each dog of Group II underwent as nearly identical a procedure as possible. After the dogs were anesthetized, an endotracheal tube was inserted to ensure a patent airway. Except for contamination from the oral cavity, a sterile technique was observed throughout the procedure.

A mucoperiosteal flap was retracted from over the area of the roots of the right mandibular posterior teeth. The teeth were sectioned with dental burs, and buccal cortical bone was removed to facilitate atraumatic removal of the teeth. The right mandibular premolars and molars were removed in each dog. An extensive alveolectomy was performed to remove all rough and jagged edges of bone and reduce the height of the ridge in order to allow close edge-to-edge approximation of the alveolar mucosa. An attempt was made to remove all tooth fragments and bone spicules by means of irrigation, curettement, and aspiration. The mucosal margins were trimmed to permit an even approximation, and the wounds were closed with interrupted, plain catgut sutures. Each dog operated upon received an intramuscular injection of procaine penicillin, 450,000 units, on the day of surgery and two days postoperatively.

Following surgery, the wounds healed by primary intention; the sutures dissolved and were exfoliated within a period of seven days, leaving clean, smooth alveolar ridges. A slight dehiscence occurred in two cases, however, which healed grossly by secondary intention within two weeks after surgery.

The animals were operated upon in groups of two at weekly intervals and were irradiated in groups of six three times per week. The wounds were allowed to heal grossly before irradiation was begun. The time interval between surgery and irradiation varied from seven to twenty-eight days and averaged sixteen days (Table I, column 3).

Irradiation Technique.—Each side of the mandible was irradiated separately through a lateral external portal. A field was used that covered the entire superior-inferior width of the mandible, extending posteriorly from the mesial aspect of the fourth premolar for a distance of 5 cm. (Fig. 1). The side of the mandible that was being irradiated was isolated with lead shields. This was accomplished by placing a lead plate, 3 mm. in thickness, on the lingual aspect of the side of the mandible being irradiated. The plate extended below the inferior border of the mandible, thereby protecting the opposite side (Fig. 2, *A*).

The cheek was partially withdrawn from the field by acrylic retractors. The portion of the mandible anterior to the field of irradiation was protected

by a leaded rubber strip (Fig. 2, *B*). This was done in order to compare the irradiated and nonirradiated areas of the same side of the mandible.

The radiation was delivered in seven equal amounts over a period of fifteen days. The total quantities ranged from 3,000 r to 8,000 r (measured in air).*

RESULTS

Gross Pathologic Findings.—Within ten to fourteen days after the course of irradiation was begun, pseudomembranous mucositis and edema developed

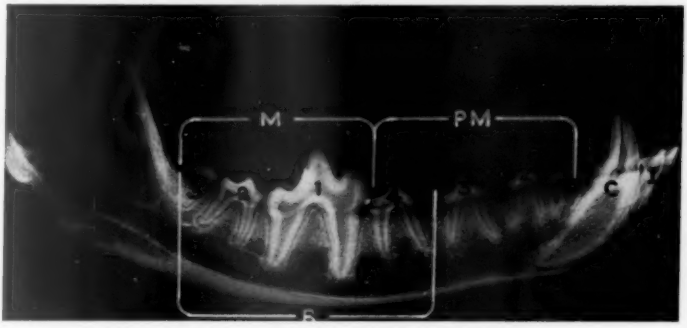

Fig. 1.—Dental formula and outline of field of irradiation. *I*, Incisors; *C*, cuspid; *PM*, premolars; *M*, molars; *R*, field of irradiation.

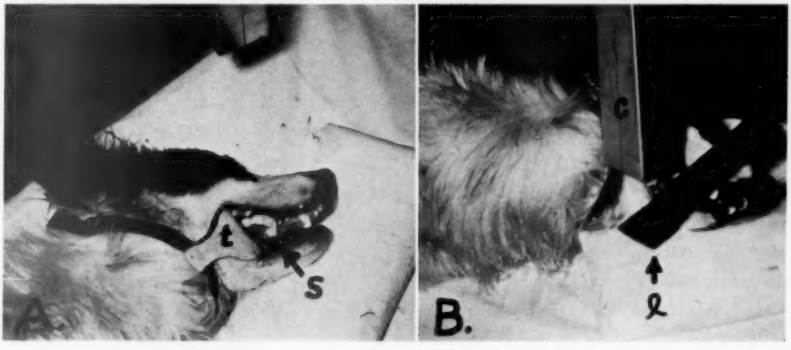

Fig. 2.—Irradiation technique.
A, Intraoral lead shield (*s*) and acrylic cheek retractor (*t*) in place.
B, Leaded rubber strip (*l*) and cone (*c*) of x-ray therapy unit in place. Animal prepared for irradiation.

in the buccal mucosa and gingiva. Depigmentation occurred in the irradiated areas at approximately the same time. Within three to four weeks, epilation

*The technical factors were as follows: 250 KVP, 30 Ma., 0.5 mm. copper filter, 1 mm. aluminum filter, with a half-value layer of 1.4 mm. of copper, TSD 50 cm., and dose rate of 105 r to 110 r per minute.

of the irradiated portion of the cheek occurred and was followed by epidermolysis in the majority of cases. The severity of the reaction varied directly with the total amount of radiation given. No gross pathologic changes were noted in the oral mucosa outside the field of irradiation. After a variable period, the reactions began to subside in the animals to which the lower quantities of radiation had been delivered. In the animals which had received the higher quantities of radiation, the reaction progressed until the soft tissue ulcerated and left the mandible exposed. Extensive ulceration of soft tissue determined the pathologic end point of the experiment.

Radiographic Findings.—In those animals of Group I and Group II, to which 4,000 r or less were delivered, no significant radiographic changes were noted in the irradiated dentulous areas. However, in those mandibles to which 5,000 r or more were delivered, widening of the periodontal membrane (especially at the apices and cervices of the teeth) and resorption of bone at the alveolar crest were noted (Fig. 3, *A*). These changes were readily discernible approximately two months after completion of irradiation.

Definite changes took place in the dentulous areas of two of the dogs in Group II. In Dogs 62 and 48, which received 6,000 r and 7,000 r, respectively, exfoliation of teeth and pathologic fractures occurred (Fig. 3, *B* and *C*). In both cases the fractures occurred in vertical, linear areas of resorption of bone at the anterior junction of irradiated and nonirradiated bone. There were also second vertical, linear areas of resorption of bone, without fracture, at the posterior border of the field of irradiation. Only slight evidence was found of resorption of bone in the central portion of the irradiated fields in Dogs 62 and 48.

In the irradiated edentulous areas of the animals in Group II, radiographic evidence of orderly reconstruction of the alveolar ridge was noted, that is, resorption of the bony walls of the alveoli and deposition of cancellous bone in the alveoli. Even in the mandible to which 8,000 r were delivered, there was evidence of bony reconstruction at the time the dog was sacrificed (Fig. 4, *A* and *B*). In general, the degree of reconstruction was inversely proportional to the amount of radiation delivered. The mandibles which received the lowest quantities of radiation showed the most reconstruction (Fig. 4, *C*). The time required for a moderate degree of alveolar bone reconstruction was approximately two and one-half months from the time of surgery.

In the mandible of Dog 48, to which 7,000 r were delivered, the alveolar bone was less dense than it was immediately after surgery; yet, there was evidence of calcification around the walls of the alveoli (Fig. 4, *D*). In the edentulous area of Dog 48 there was very little evidence of resorption of bone, either generalized or at the border of the irradiated field, as compared to the dentulous side of the mandible (Fig. 3, *C*).

Autopsy Findings.—At autopsy the alveolar mucosa remained intact in the irradiated areas in those animals of Group I to which 4,000 r or less were

delivered (except in Dogs 83 and 134, in which the teeth were removed following irradiation). Large ulcerations occurred bilaterally in the mucosa, exposing the mandibles, in the areas to which 5,000 r or more were delivered (Fig. 5, *A*).

In Group II, ulceration of the alveolar mucosa was noted in twelve of the fourteen areas that had been irradiated with teeth present. Ulceration occurred in all dentulous areas in the dogs of Group II that received 4,000 r or more,

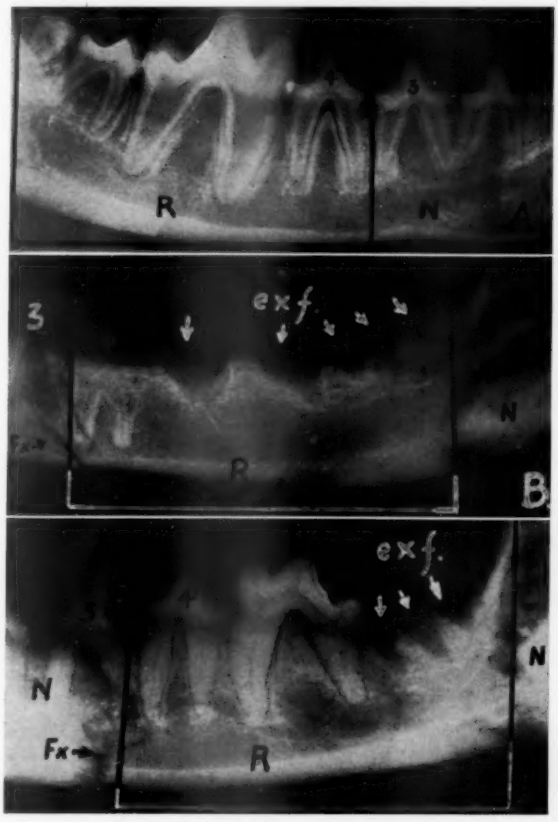

Fig. 3.—Radiographs of dentulous autopsy specimen illustrating the pathologic changes. *A*, The right side of the mandible of Dog 185, Group I, which was exposed to 5,000 r, illustrates the condition of the teeth and bone forty-one days following irradiation (see Table II). Note the radiolucent areas at the apices of the teeth within the irradiated field (*R*) and the distal root of the third premolar (*3*) which also received some radiation. The architecture of the bone at the apices of the teeth in the shielded anterior area (*N*) appears normal radiographically. By histologic study it was confirmed that osteomyelitis was present in the entire irradiated area (R) but absent in the anterior portion of the nonirradiated area (*N*).

There was a progression of the pathologic process in two of the dentulous mandibles in Group II, Dog 62 (*B*) and Dog 48 (*C*) which were exposed to 6,000 r and 7,000 r respectively (see Tables I and III). In both of these specimens there was destruction of the periodontal membranes, leading to exfoliation (*exf.*) of teeth. There were vertical linear areas of resorption of bone immediately outside the borders of the irradiated fields, with pathologic fractures (*Fx*) at the anterior borders. There was very little evidence of resorption of bone within the irradiated fields (R).

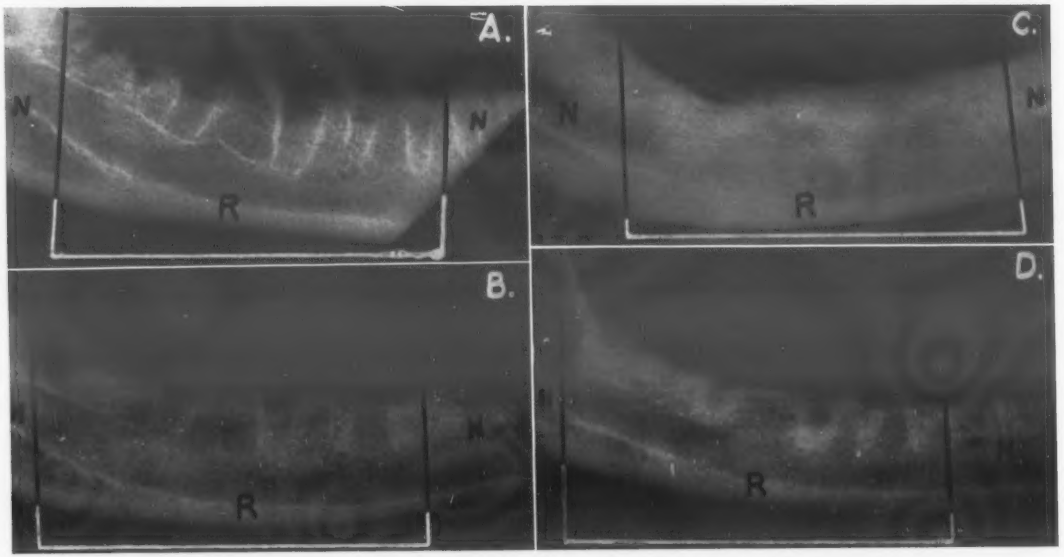

Fig. 4.—Radiographs of edentulous specimens illustrating the degree of alveolar reconstruction.

Radiographs of Dog 52, which was exposed to 8,000 r, illustrating the changes in the bone from immediately after surgery (*A*) to seventy-one days and forty-nine days following surgery and irradiation, respectively (*B*) (see Tables I and III).

In *B*, compare the density of the alveoli in the irradiated (*R*) and nonirradiated (*N*) areas.

C is a radiograph of the autopsy specimen of Dog 75, which was exposed to 4,000 r, illustrating good alveolar reconstruction which occurred in the low-dosage range.

D is a radiograph of the autopsy specimen of Dog 48, which was exposed to 7,000 r, illustrating moderate alveolar reconstruction. Compare *D* with Fig. 3, *C* which is the opposite dentulous side of the same mandible. Note in *D* the lack of resorption of bone and the absence of pathologic fracture. Histologic studies revealed massive osteomyelitis in the dentulous side (Fig. 3, *C*) but no osteomyelitis in the edentulous side (*D*) (see Tables I and III).

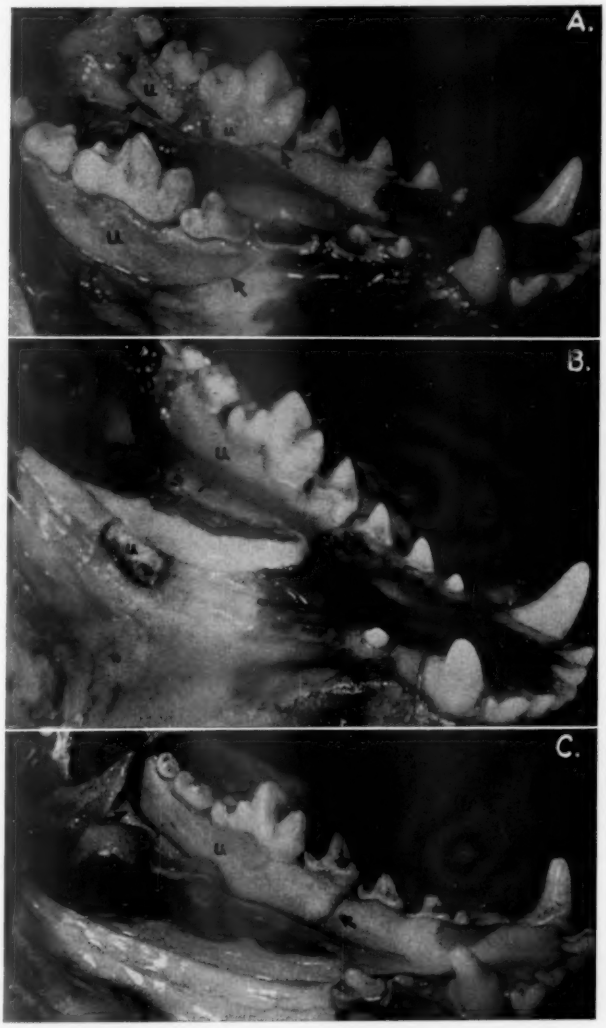

Fig. 5.—Photographs of autopsy specimens illustrating the degree of mucosal ulcerations. *A*, Dog 185, represents the gross pathologic condition of the four specimens of Group I which were exposed to 5,000 r or more and illustrates the bilateral ulcerations (*u*) which occurred with teeth present (see Table II).

B, Dog 16, which was exposed to 6,000 r, illustrates the condition seen in a few instances in Group II wherein there were ulcerations (*u*) in both the dentulous and the edentulous areas. Compare the small area of ulceration (*u*) on the edentulous side with the much larger area on the dentulous side (see Tables I and III).

C, Dog 157, which was exposed to 5,000 r, is a representative sample of eight similar specimens illustrating a smooth, clean, intact mucosa on the edentulous side and a large ulceration (*u*) on the dentulous side. With only two exceptions in the entire series of animals, an ulceration was accompanied by osteomyelitis (see Table III, Dogs 16 and 80, compare right and left sides). Osteomyelitis was not present where the mucosa remained intact.

with the exception of Dog 105. Ulcerations of the alveolar mucosa took place in five of the fourteen irradiated edentulous areas (Tables I and III).

The ulcerations in the edentulous areas were relatively small compared to those in areas in which teeth were present (Fig. 5, *B*). Ulcerations in the dentulous areas were consistently large and produced orocutaneous fistulas in a few cases. In all instances, the ulcerations in the edentulous areas were localized to the alveolar ridge with the exception of Dog 105, in which the ulceration originated in the cheek and spread to the alveolar ridge.

The most notable results were seen in the eight dogs in which the mucosa of the edentulous areas remained intact while large ulcerations developed in the areas where teeth were present (Fig. 5, *C*).

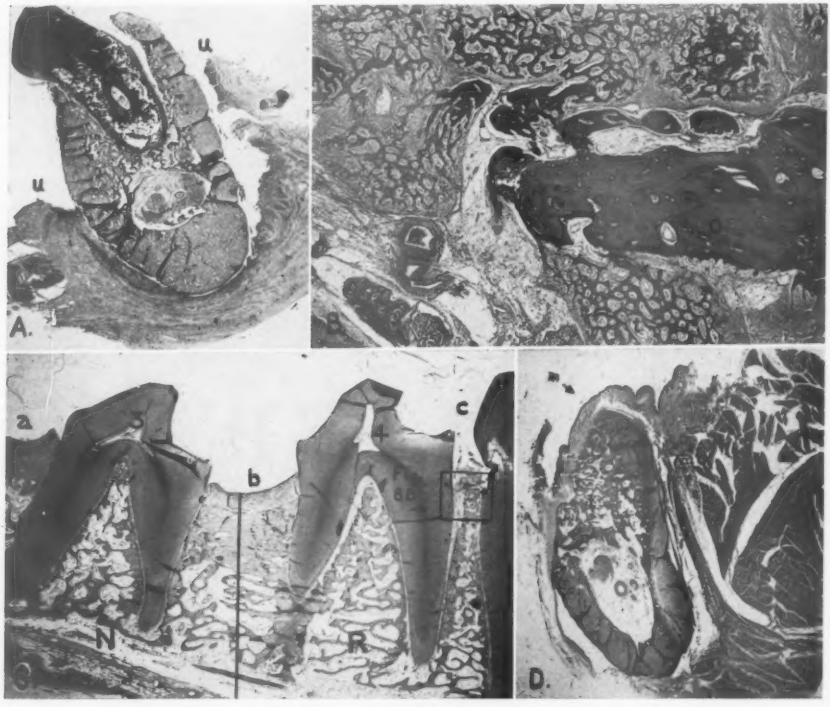

Fig. 6.—Photomicrographs of sections stained with hematoxylin and eosin illustrate the condition of the bone and soft tissue following x-ray irradiation.

A, Dog 80, which was exposed to 6,000 r. This cross section illustrates at low-power magnification the massive ulcerations (*u*) seen grossly in Fig. 5, *A*, *B*, and *C*, the inflammation (*i*) of the perimandibular soft tissues, and the extensive osteomyelitis that resulted.

B, Dog 185, which was exposed to 5,000 r. This cross section illustrates the resorption of old bone (*o*) and the deposition of new trabecular bone (*t*) seen in the areas where osteomyelitis occurred.

C, Dog 84, which was exposed to 6,000 r, illustrates the transition from the nonirradiated (*N*) to the irradiated (*R*) area. Note the transition of the mucosa from normal (*a*), to atrophic (*b*), to necrotic (*c*). Note the normal pulp in the third premolar (*3*) and obliteration of the pulp in the fourth premolar (*4*).

D, Dog 76, which was exposed to 5,000 r, illustrates the intact alveolar mucosa (*m*), the new trabecular bone (*t*) filling the alveolus and the lack of evidence of osteomyelitis. This cross section represents the microscopic findings seen in the mandibles illustrated in Fig. 5, *C*. Compare *D*, an edentulous area, with *A*, a dentulous area.

TABLE I. GROUP II. TIME SEQUENCE BETWEEN SURGERY, IRRADIATION, DETECTION OF ULCERATIONS, AND SACRIFICE OF DOGS

DOG NO.	TOTAL IRRADIATION (R)	TIME (DAYS): SURGERY TO IRRADIATION	TIME (DAYS): END OF IRRADIATION TO DETECTION OF ULCERATION		TIME (DAYS): END OF IRRADIATION TO SACRIFICE OF DOG
			LEFT (TEETH PRESENT)	RIGHT (EDENTULOUS)	
195	3,000	7	No ulceration	No ulceration	400+
75	4,000	22	49	33	50
188	4,000	21	99	No ulceration	100
18	4,000	21	48	No ulceration	50
105	4,000	28	No ulceration	330	361
76	5,000	22	49	No ulceration	72
77	5,000	19	32	No ulceration	50
157	5,000	21	40	No ulceration	65
16	6,000	7	33	33	50
62	6,000	9	36	No ulceration	57
80	6,000	14	77	84	85
48	7,000	9	67	No ulceration	122
78	7,000	19	33	No ulceration	50
52	8,000	9	57	57	57

Histopathologic Findings.—The histopathologic findings correlated very closely with the gross pathologic findings. In each instance in Group I and in the dentulous areas of dogs in Group II there was a concomitant acute suppurative osteomyelitis when ulcerations occurred in the mucosa (Fig. 6, *A*). In these areas the inflammatory response was moderate to severe (Fig. 6, *B*).

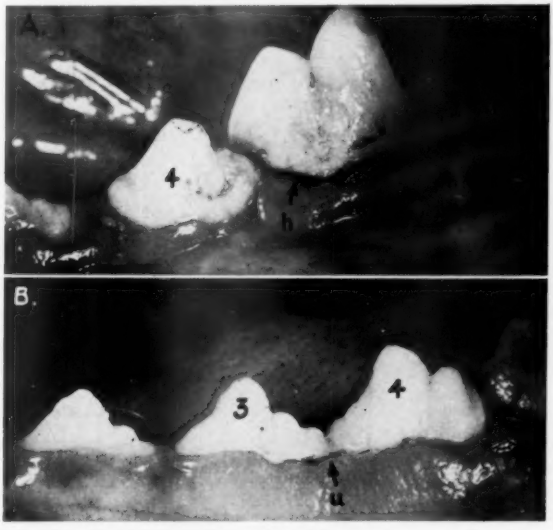

Fig. 7.—Gingivitis following irradiation.

A, Dog 76, which was exposed to 5,000 r, illustrates the hemorrhagic gingivitis (*h*) which was present one month post-irradiation. This was a consistent finding in all of the irradiated dentulous areas.

B, Dog 78, which was exposed to 7,000 r, illustrates the ulcerative gingivitis which was present one month postirradiation. This was a consistent finding in all of the irradiated dentulous areas that were exposed to 5,000 r or more. Once the point of ulceration occurred, the gingivitis did not heal but progressed to massive mucosal ulcerations. Note the ulceration (*u*) in the interproximal area between the third (*3*) and fourth (*4*) premolars. In all instances, this was the first area to become ulcerated.

Resorption of old bone and deposition of new bone were seen in the areas of osteomyelitis in which the teeth were present. This occurred even in the areas to which 8,000 r had been delivered.

No histopathologic changes were noted in the pulps of teeth that received 4,000 r or less. However, the pulps of teeth that received 5,000 r or more became hyperemic, edematous, and infiltrated with inflammatory cells. The pulps that received the higher amount of radiation became necrotic and were obliterated (Fig. 6, *C*).

In the edentulous areas of dogs in Group II where ulcerations occurred in the mucosa, there was a concomitant osteomyelitis in all instances, with the exception of Dogs 16 and 80. The ulcerations were very small in these dogs, and the inflammation remained localized to the surface of the bone. Otherwise, the degree of inflammatory response varied directly with the size of the ulcerated area. In the edentulous fields in which the areas of ulceration were large (Dogs 75, 105, and 52), the inflammatory response was severe. There

TABLE II. GROUP I. CONDITION OF ALVEOLAR MUCOSA AND BONE AT AUTOPSY

DOG NO.	TOTAL IRRADIATION (ROENTGENS)	TEETH PRESENT BILATERALLY DURING BILATERAL IRRADIATION			
		LEFT MANDIBLE		RIGHT MANDIBLE	
		ULCERATION OF ALVEOLAR MUCOSA	OSTEOMYELITIS	ULCERATION OF ALVEOLAR MUCOSA	OSTEOMYELITIS
120*	3,000	0	0	0	0
83*	4,000	0	0	+++	++++
134*	4,000	0	0	+++	++++
82	5,000	++++	++++	++++	++++
185	5,000	++++	++++	++++	+++
84	6,000	++++	++++	++++	++++
58†	7,000				
60	8,000	++++	++++	++++	++++

*In these dogs the right posterior teeth were removed subsequent to irradiation. The effect of removal of teeth following irradiation is now being studied.
†Dog 58 died during the course of irradiation.

TABLE III. GROUP II. CONDITION OF ALVEOLAR MUCOSA AND BONE AT AUTOPSY

DOG NO.	TOTAL IRRADIATION (ROENTGENS)	TEETH PRESENT DURING IRRADIATION (LEFT)		TEETH REMOVED BEFORE IRRADIATION (RIGHT)	
		ULCERATION OF ALVEOLAR SOFT TISSUE	OSTEOMYELITIS OF MANDIBLE	ULCERATION OF ALVEOLAR SOFT TISSUE	OSTEOMYELITIS OF MANDIBLE
195	3,000	0	0	0	0
75	4,000	++	++	++++	++++
188	4,000	++++	++	0	0
18	4,000	++++	++	0	0
105	4,000	0	0	+++	+++
76	5,000	++++	++++	0	0
77	5,000	+++	++	0	0
157	5,000	++++	++++	0	0
16	6,000	++++	++++	+	0
62	6,000	++++	++++	0	0
80	6,000	++++	++++	±	0
48	7,000	++++	++++	0	0
78	7,000	+++	++	0	0
52	8,000	++++	++	++	++

was no osteomyelitis in the edentulous fields in which the mucosa remained intact (Fig. 6, *D*). In every area from which the teeth had been removed before irradiation, new spongy bone was present in the alveoli, even in the mandible to which 8,000 r had been delivered.

The histopathologic findings are summarized in Tables II and III.

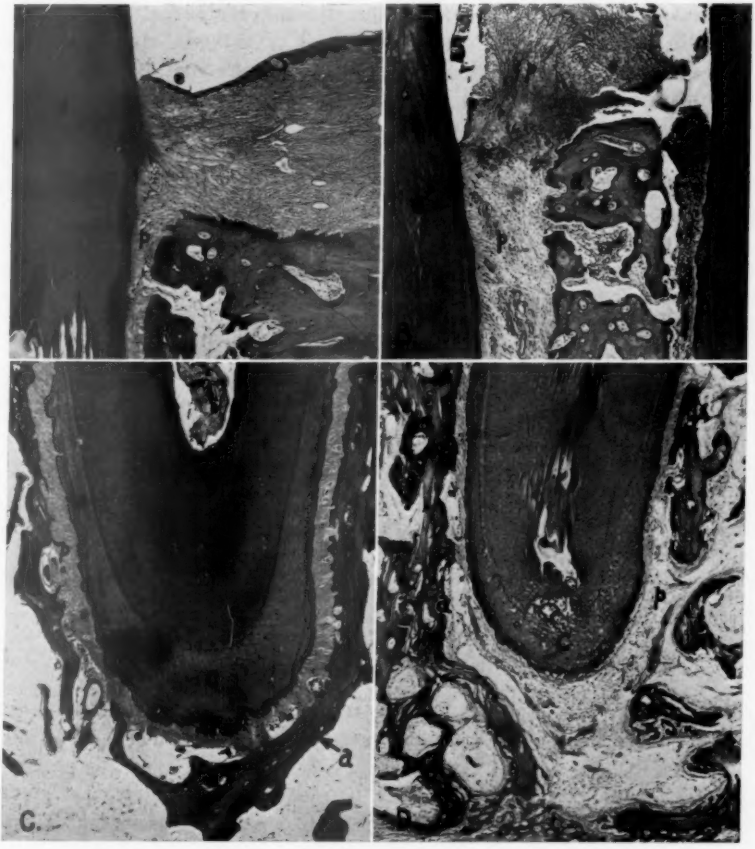

Fig. 8.—Comparison of photomicrographs of normal control and irradiated cervical and apical areas of the teeth.

A, The normal cervical area, illustrates the epithelial attachment (*e*), periodontal membrane (*p*), and alveolar bone (*a*). Note the thinness of the epithelial attachment near the tooth (*d*).

B, The irradiated cervical area of Dog 84, which was exposed to 6,000 r, illustrates a fibrinopurulent exudate (*fp*) replacing the epithelial attachment and interproximal mucous membrane. There is marked inflammation of the periodontal membrane (*p*) superficially and extension of the inflammatory reaction into the adjacent alveolar bone (*a*).

C, The normal apical area, illustrating a prominent zone of cementum (*c*), a thin, dense periodontal membrane (*p*), and an intact alveolar bone (*a*).

D, The irradiated apical area of Dog 185, which was exposed to 5,000 r, illustrates the inflammatory dissolution of the periodontal membrane (*p*), resorption of alveolar bone (*a*), and deposition of new trabecular bone (*t*) within the medullary shaft. In most instances the periodontal membrane was affected in the cervical and apical areas with the intervening portion relatively uninvolved, indicating that the inflammation progressed from the cervical to the apical area through the medullary bone.

Pathogenesis.—In the irradiated areas in which teeth were present, the mucosal reactions were the same as in the edentulous areas. When the teeth were present, however, a concomitant hemorrhagic gingivitis was noted (Fig. 7, *A*). In the areas to which 5,000 r or more in Group I and 4,000 r or more in Group II were delivered, the hemorrhagic gingivitis progressed to an ulcerative gingivitis (Fig 7, *B*). When the point of ulceration had been reached, the gingiva did not heal and the process continued until the alveolar mucosa ulcerated, leaving the mandible exposed over a large area. These ulcerations progressed to orocutaneous fistulas exposing the inferior surface of the mandible in some of the dogs to which the higher quantities of radiation had been delivered.

Radiographs showed widening of the periodontal membrane (especially at the apices and cervices of the teeth) and resorption of bone at the alveolar crest (Fig. 3, *A*). This was confirmed by histopathologic examination. The first area involved in the inflammatory process appeared to be the region of the epithelial attachment (Fig. 8, *A* and *B*). The lysis of this tissue apparently offered the means of entry for microorganisms.

After the soft tissue near the cervix of the tooth ulcerated, the inflammatory process spread diffusely through the medullary bone within the field and for variable distances beyond the borders of the irradiated field. The inflammatory process never spread to the opposite side of the mandible.

The periodontal membrane appeared to be relatively uninvolved in the inflammatory process except in the most advanced cases. In some instances the epithelial attachments were destroyed and the medullary bone was involved extensively, but with the process spreading into the periodontal membrane only at the cervices and apices of the teeth (Fig. 8, *C* and *D*).

It was not determined where the necrosis and ulceration started in the edentulous areas, or what acted as the predisposing and initiating factors.

DISCUSSION AND COMMENTS

In Group I, in which all teeth were present at the time of irradiation, osteomyelitis developed in every instance where the quantity of irradiation was 5,000 r or more (Table II).

Suitable analytic measures were applied to determine the statistical validity of the results obtained in Group II, in which dentulous and edentulous areas could be compared. Osteomyelitis occurred in twelve of thirteen dentulous areas to which 4,000 r or more were delivered; however, osteomyelitis occurred in only three of thirteen corresponding edentulous areas. This difference was statistically valid, since the probability of chance occurrence would be less than 5 per cent.[45] Therefore, the incidence of osteomyelitis was reduced by the removal of teeth before irradiation.

The question then arises as to why the presence of teeth at the time of irradiation increased the incidence of mandibular osteomyelitis. This experiment was not designed to elucidate that answer, and therefore it offered no solution. The experience gained in conducting it, however, may permit the consideration of some factors for speculation.

The difference in results may have been caused by a variety of factors, such as difference in vascular changes, alteration in cellular response initiated by the operative procedure, difference in masticatory trauma, variation in thickness of the epithelial attachment as compared to the thickness of the epithelium of intact alveolar mucosa, difference in quantity of secondary radiation, etc.

Although all of these factors may have been contributory, some were undoubtedly more significant than others. As far as vascular changes were concerned, for instance, no obliterating endarteritis was detected in either side of the mandibles, possibly because of the short duration of the experiment.

As for masticatory trauma, the diet was of a consistency that could be ingested without mastication. However, the dogs were able to bring their teeth into occlusion, which could have added some stress to the periodontal tissues of the dentulous side. In addition, the presence of gingival crevices would invite the impaction of food and the accumulation of debris, which may have served as a nidus for infection. The irregularity of the dentulous anatomic situation contrasted very notably with the smooth contour of the mucosa in the edentulous areas.

Next, we may consider the difference in thickness of the epithelium between the dentulous and edentulous areas. Although the epithelium in the irradiated edentulous areas was atrophic and thinner than in the nonirradiated edentulous areas, it was still much thicker than the epithelial attachment. The area of the epithelial attachment appeared to be the most vulnerable region in the irradiated dentulous field.

The matter of difference in the quantity of secondary radiation occuring in the dentulous and edentulous areas would appear to be a significant factor. It has been stated that the periosteum is greatly affected by secondary radiation, due to the high calcium content of bone.[7, 30] The calcium content of tooth structure is higher than that of bone per unit volume; furthermore, the epithelial attachment bears the same intimate relationship to the tooth as the periosteum does to the bone. Thus, it might be expected that the epithelial attachment would receive a higher total quantity of radiation as a result of secondary radiation. It has been estimated that the effect of secondary radiation from the teeth would be manifested mainly within 1 or 2 mm. from the teeth and that, as a result of tooth contour, the greatest quantity would be received in the interproximal areas.[46] The interproximal area was the site in which the initial ulceration was observed in each case. It would appear, therefore, that secondary radiation was a predisposing factor in the production of osteomyelitis in the dentulous areas. Further study is necessary to prove or disprove this point.

SUMMARY AND CONCLUSIONS

1. An experimental method for surgical removal of the teeth and controlled irradiation of the mandibles of dogs has been described.

2. Mandibular osteomyelitis did not occur in dogs following irradiation when an intact alveolar mucosa was present.

3. There was a moderate degree of reconstruction of the edentulous alveolar process following irradiation of the dog mandibles up to a quantity of 8,000 r.

4. Removal of the teeth shortly before irradiation of the dog mandibles significantly reduced the incidence of osteomyelitis.

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EXODONTIA

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IMPACTED MANDIBULAR THIRD MOLAR COMPLICATED BY THROMBOCYTOPENIC PURPURA

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DISEASES of the blood or blood-forming tissues are of particular interest to the oral surgeon. The abnormalities produced by these diseases complicate any surgical procedure and may at times present a real threat to the patient's recovery. This report, dealing with a specific threat, is of particular interest from the viewpoint of management.

Thrombocytopenic purpura, also called purpura hemorrhagica and Werlhof's disease, was first described by Werlhof in 1740.[1] The chief clinical manifestation is hemorrhage, spontaneous or traumatic, into the skin and muscles or from the mucous membranes of the nose, oral cavity, gastrointestinal, and genitourinary tracts. Hemorrhage into the central nervous system is to be feared.[2] Hematuria may be present, and the spleen may be enlarged. The Rumpel-Leede test, which involves application of a tourniquet to the upper arm inflated to the mean value between systolic and diastolic pressures so as to obstruct venous but not arterial blood flow and held there for five minutes, causes petechial hemorrhages to appear in the skin below the tourniquet.[3] A purpuric response is observed by the appearance of petechia in excess of 15 in a circle one inch in diameter. The bleeding time is greatly prolonged, whereas the clotting time is usually normal. Clot retraction is poor. As the name of this disease implies, thrombocytopenia is the finding in examination of the blood. In this disease the platelet count falls below 60,000 per cubic millimeter (as compared to the normal count of 200,000 to 400,000 per cubic millimeter) and may in severe cases disappear completely.

"The main symptom is the hemorrhage, the main lesion thrombocytopenia."[4] This disease may be classified as primary or essential, with an unknown etiology, or secondary, resulting from infections, tumors, radioactive

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substances, or chemical or drug idiosyncrasies. The primary form is usually seen in the young, while the secondary form is more common in the adult.[5] These classifications are subdivided into acute and chronic types. The acute type is usually fatal; the chronic type extends for years. Remissions may occur in both the primary and the secondary forms, with disappearance of all symptoms. Splenectomy is of value in the chronic cases, in which event it will result in a marked rise in the blood platelets. Although this cell increase is not permanent, the purpuric manifestations do not necessarily return.

Surgery performed on a patient affected with this disease carries an obvious hazard of uncontrolled hemorrhage, and a successful outcome is dependent on proper management. A case involving elective oral surgery on a patient affected with thrombocytopenic purpura is reported. The patient was referred for treatment of an infection in the right mandible.

Complaint and History of Present Illness.—A 27-year-old white man was first seen on Oct. 20, 1956, with the complaints of recurrent pain and swelling of four days' duration in the mandibular right third molar area, with painful movements of the jaw. These were recurrent symptoms that had first been noticed approximately six months previously. The latest episode was the most severe.

Past History.—The patient reported that he had received the diagnosis of thrombocytopenic purpura several years before in New York City when purpuric symptoms had caused him to undergo a physical examination. Except for easy bruising, there had been no alarming purpuric manifestations in the past year, and "tests one year ago were negative." Family history was negative for purpura.

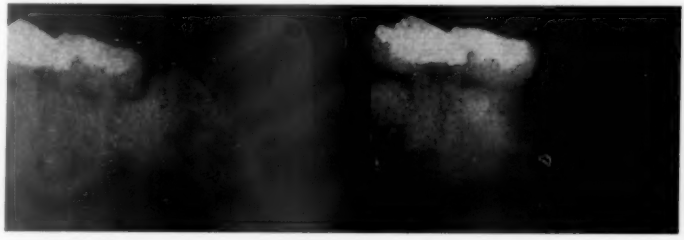

Fig. 1. Fig. 2.

Fig. 1.—Oct. 20, 1956. Preoperative roentgenogram demonstrating mesio-angular impacted mandibular right third molar.
Fig. 2.—Nov. 6, 1956. Postoperative roentgenogram showing removal of the third molar.

Examination.—The soft tissue overlying the angle of the right mandible was indurated, and the submaxillary and cervical lymph nodes draining this area were palpable and tender. A moderate degree of trismus was present. Intraorally, the dentition was essentially normal except for the third molars, which were not to be seen. In the mandibular right third molar region there was considerable inflammation. A small amount of purulent exudate was draining from the inflamed tissue just distal to the second molar. At this point of exit there was an area, approximately 1 mm. in size, from which slight capillary hemorrhage was evident. The patient's oral temperature was 100.1° F. An intraoral periapical roentgenogram of the mandibular right molar area revealed a mesio-angular impacted third molar (Fig. 1).

A diagnosis of impacted mandibular right third molar with nonspecific pericoronitis complicated by and manifesting evidence of thrombocytopenic purpura was made. The continuous, though minimal, bleeding from the small area in the third molar region was thought

to be an oral manifestation of the systemic condition. Penicillin, 1,200,000 units,* was given intramuscularly, frequent warm rinses were prescribed, and the patient was referred to an internist for physical examination and evaluation of the blood dyscrasia.

Consultation Report.—The patient was seen by the internist on Oct. 22, 1956. A physical examination with pertinent laboratory procedures was performed. The maxillofacial and oral symptoms had abated markedly, and the patient's oral temperature was normal. The spleen was of normal size. A Rumpel-Leede test was negative. The results of the laboratory procedures performed on that day were as follows: white blood cells, 6,700; hemoglobin, 14 grams; hematocrit, 44.5 per cent; differential—segmented 82, total polymorphonuclear leukocytes 82, lymphocytes 14, and eosinophils 4, platelets greatly reduced; sedimentation rate 4 mm. per hour (Wintrobe); bleeding time 7 minutes 30 seconds. There was no clot retraction in twenty-four hours, with a mushy clot present.

A diagnosis of thrombocytopenic purpura was established. As the patient was a chemist, the thought was entertained that this was a secondary form caused by chemical intoxication. It was concluded that the patient was enjoying a period during which elective oral surgery could be undertaken, provided the patient was hospitalized and whole blood was held in readiness for immediate transfusion. It was decided to withhold a preoperative transfusion and any postoperative administration unless necessary, because of the inherent dangers of blood administration. For example, Cecil and Loeb[6] state: "The incidence of hepatitis among those receiving transfusions exceeds 3 per cent in certain clinics despite careful questioning and laboratory screening of all donors."

Treatment and Course.—The patient was admitted to Madison General Hospital on Oct. 30, 1956, and prepared for surgery on the following day. On admission, the temperature, pulse, and respirations were normal; blood pressure was 118/66. A physical examination performed by the resident on duty revealed a positive Rumpel-Leede test with twenty-five or more petechiae counted. The internist confirmed this report and ordered preoperative laboratory examinations, which revealed the following:

Urinalysis:

Color: straw—clear. Reaction: acid. Specific gravity: 1.026. Negative albumin, glucose, acetone. Pus: occasional clumps, high-power field. Red blood cells: 0-5, high-power field.

Hematology:

Hemoglobin 13.8 grams per 100 c.c., 89 per cent. Hematocrit: 39.

Red blood cell morphology: anisocytosis. white blood cells: 8,150 per cubic millimeter.

Differential: 82 neutrophils; 0 basophils; 1 eosinophil; 12 lymphocytes; 5 mononuclear leukocytes.

Platelets: 36,000 per cubic millimeter. Bleeding time: 5 minutes 20 seconds. Clot retraction: Clot begins at three hours. Wassermann: negative.

Whole blood was cross-matched and typed, and 500 c.c. was held in readiness. The author ordered Seconal sodium, ¾ gr., and Amytal sodium, ¾ gr., to be given at bedtime. Procaine penicillin, 400,000 units, was ordered given that day and to be repeated the next morning prior to surgery. The patient was advised to brush his teeth and rinse his mouth just prior to surgery in the morning. The patient was prepared for the general anesthesia by the anesthesiologist, who ordered abstinence from food and liquid after midnight and ⅙ gr. morphine with $\frac{1}{200}$ gr. scopolamine by hypodermic at 7 A.M. on Oct. 31, 1956.

On Oct. 31, 1956, the patient was brought to the operating room, and induction of the general anesthesia was begun by the anesthesiologist at 8 A.M. Anesthesia was induced with thiopental sodium, 350 mg., with succinylcholine chloride, 20 mg., added. A cuffed oral-endotracheal tube was introduced slowly and carefully to avoid bruising. Anesthesia was

*Benzathine penicillin, 600,000 units; procaine penicillin, 300,000 units; and potassium penicillin, 300,000 units.

maintained without event with nitrous-oxide-oxygen and trichloroethylene using a semiclosed system. A mouth prop was inserted and the mouth was opened. Suction was used to cleanse the oropharynx, and moist 4 by 8 inch gauze was placed so as to occlude the pharynx.

With a No. 15 blade, an incision was made in the buccal gingival sulcus from the distal side of the first molar posteriorly into the retromolar triangle. A mucoperiosteal flap was retracted, exposing the impacted third molar. Care was exercised at all times to avoid undue trauma. With a sharp chisel and hand mallet, the overlying bone was removed from the buccal and alveolar surfaces, exposing the crown of the impacted molar. With a No. 703 burr under saline irrigation, the crown was sectioned and delivered. The burr was again used to section the roots, which were delivered one at a time. The edges of the bony defect were rounded with a suitable file and the surgical area thoroughly débrided. A small piece of Gelfoam was inserted, and the wound was closed with interrupted 000 silk sutures. One suture was placed between the first and second molar, and three were placed over the third molar area. Hemorrhage was not a problem at surgery. At 9 A.M. the anesthesia was discontinued and the patient was taken to the postoperative recovery room.

When the patient had regained consciousness and was returned to his room, the head of his bed was elevated 45 degrees and an ice bag was applied to the right maxillofacial region. It was advised that he bite on a 2 × 2 gauze placed over the wound and changed every half-hour. Mouthwashes were not permitted and, except for bathroom privileges, complete bed rest was ordered. APC compound with codeine phosphate, 0.25 gr., every four hours if necessary for relief of pain, was prescribed. Postoperative blood pressure at noon was 158/100. It declined to 128/72 at 8 P.M. Respirations remained normal at 18 to 20 per minute.

The following day, Nov. 1, 1956, considerable edema had developed, extending from the zygoma into the submaxillary area on the right side. Intraorally, the degree of edema was similar and steady, although minimal capillary hemorrhage was evident. The patient was reasonably comfortable. The immediate postoperative orders regarding body position, cold therapy, etc. were continued.

On the morning of Nov. 2, 1956, the second postoperative day, the patient was more comfortable although the intraoral and extraoral edema persisted. The capillary hemorrhage was about the same as on the first postoperative day. On the afternoon of that day the author was notified by the resident that more active hemorrhage from the oral wound had begun. It was evaluated to be of a minimal magnitude, so the transfusion was withheld. By 7 P.M. the hemorrhaging had subsided to the rate that had existed during the morning. An examination of blood drawn the previous day revealed the following: Platelets: 43,000 per cubic millimeter, clot retraction began at four hours; retracted after twenty-four hours, but still not as complete as normal retraction.

The third postoperative day, Nov. 3, 1956, found the patient markedly improved, both physically and mentally. The edema was subsiding and the capillary hemorrhage had diminished to spotting. Ecchymosis appeared on this day in the skin over the angle of the mandible on the right side. The patient was discharged with instructions regarding home care and was requested to report to the office in three days for a follow-up examination. The internist advised that the patient be seen for further studies and investigation regarding a splenectomy.

The patient reported for an office examination on Nov. 6, 1956. The edema had almost completely subsided, and the ecchymosis had begun to fade. Intraorally, the tissue over the wound was still somewhat spongy. As the sutures were removed, some bleeding occurred. A postoperative roentgenogram (Fig. 2) revealed complete removal of the impacted third molar. Further recovery was uneventful.

SUMMARY

The pathology to be seen in thrombocytopenic purpura is discussed with equal emphasis on the clinical and laboratory findings. To the oral surgeon

the chief interest in this disease lies in the threat of prolonged hemorrhage. The case of a patient with an impacted mandibular third molar complicated by pericoronitis and thrombocytopenic purpura has been presented. The importance of close cooperation with an internist is stressed, with results of clinical and laboratory findings. The preoperative office and hospital studies are reported. Whole blood for transfusion was held in readiness. Anesthesia and surgical procedures are detailed with care to avoid unnecessary trauma. The postoperative care is given close attention. Awareness of the responsibilities involved, a high state of preparedness, and assiduous execution of a plan of treatment are believed to have been vital to the successful outcome of this case.

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214 W. WASHINGTON ST.

ORAL MEDICINE

SCLERODERMA

Its Oral Manifestations

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MODERN dentistry, like modern medicine, is fast becoming a science of protean facets. The concept of dentistry as being strictly biomechanical has been (or should be) discarded. The dentist, as guardian of the oral cavity, must accept the responsibility of recognizing systemic as well as local diseases and of then channeling them to the source of proper treatment. It is common knowledge that myriad systemic diseases manifest themselves in the oral cavity. Progressive systemic sclerosis or scleroderma is one of this large group.

Recent publications[3, 4, 8-10] have pointed out that in scleroderma one is dealing with a systemic disease which neither solely nor primarily involves the skin. Scleroderma is but a striking symptom of a generalized disease. The more serious symptoms arise in the various viscera of the body. The descriptive term *progressive systemic sclerosis* was advanced by Goetz[9] in 1945 "as describing the condition more adequately until such time as the cause has been established."

Progressive systemic sclerosis was first mentioned by Zacterus in 1634[15] and first described by Curzio of Naples in 1752.[13] Gintrac suggested the name scleroderma in 1847.[13]

The disease occurs at any age and affects females more often than males. The most common, and often unrecognized, form of progressive systemic sclerosis is a localized area of thickened skin which adheres to the subcutaneous tissues and presents a smooth, waxy surface with progression to atrophy. This form, morphea or localized scleroderma, is usually of slight consequence. A case reported by Taylor[22] is suggestive of this form. The skin over the angle and part of the body of the mandible was affected, causing resorption of

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the adjacent mandible over a five-year period. A similar case involving mucous membrane was recorded by Barber.[2] In that case a sharply circumscribed, depressed, indurated white patch extended from the vermilion border to the alveolar sulcus of the lower left lip. This was present for six months and resolved after the patient recovered from a period of great mental stress.

Morphea is by far the least serious form of the disease unless a joint is directly affected. In children morphea can affect the growth of the underlying muscle, producing deformity. Occasionally, indurated depressed bands occur in the midline of the forehead. These may resemble a saber cut; hence the term *coup de sabre*.

Fig. 1.—Case 1. Sclerodactylia. Note tapering of fingers, atrophy of the epidermis, and crippling deformities.

Diffuse progressive systemic sclerosis is a serious disease afflicting both men and women and occasionally children. The skin changes are more widespread and steadily progressive. The onset may be gradual and insidious with a chronic course, or it may occur in an acute process. Pain, swelling, and stiffness of the joints are the usual early symptoms and are often mistaken for arthritis, as seen in both of the cases to be discussed. When the fingers become sclerodermatous (Fig. 1) the term "sclerodactylia" is used. Vasomotor symptoms are present in approximately 30 per cent of the patients at the onset of the disease.

In this form the extremities and much of the trunk are usually involved. The lines of expression and wrinkles of the face are absent, producing a mask-like facies. The skin is taut and firmly bound to the underlying tissue. This

produces a pinched appearance over the bridge of the nose. Mouth movements are handicapped and the lips are retracted, preventing adequate exposure of the oral cavity. The buccal cul-de-sac is often obliterated because of the tightness of the overlying skin. A circumoral puckering and pallor occur when the mouth is opened widely. This is on a mechanical basis. In fact, limitation of mouth opening may be the initial complaint. Characteristically, vitiligo, erythema, or hyperpigmentation is apparent on the involved skin and may be the initial sign. Pigmentation of the mucous membrane is rare,[4] but it has been reported.[20] Furthermore, the skin is difficult to pick up between the fingers. Chronic ulceration of the atrophied epidermis is a frequent finding. Most of the body becomes involved within a two-year period, and remissions are rare.

Among patients with generalized disease it is possible to separate a group in whom skin changes are limited to the face, upper chest, hands, and forearms. Whether this form, acrosclerosis, is a disease entity in itself or merely a manifestation of progressive systemic sclerosis is open to dispute.[5, 9, 16] Acrosclerosis is commonly preceded by Raynaud's syndrome and followed by sclerodactylia, it is most common in female patients. The prognosis for life is favorable and an arrest of local changes is not uncommon. In this form, visceral lesions are most frequent. Dysphagia associated with esophageal involvement is a frequent finding.

ETIOLOGY

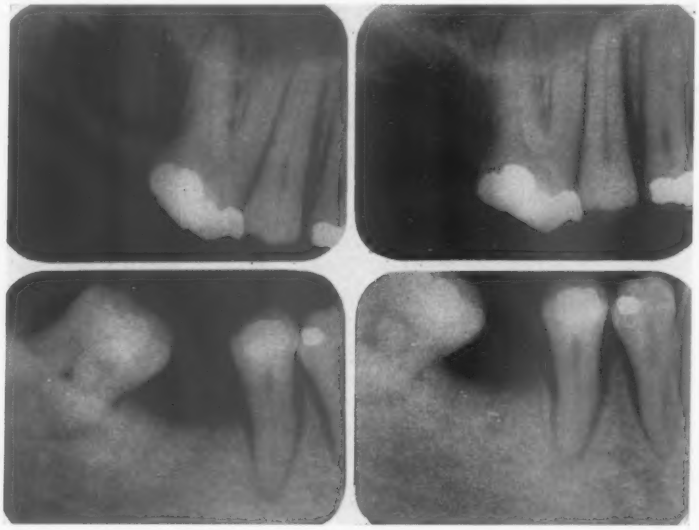
The cause and mechanism of progressive systemic sclerosis are unknown. Mental strains, overexposure to sunlight or cold, drug intoxications, and acute infections have been cited. An antigen-antibody allergic type of reaction has recently been postulated.[5] "The comparative mildness of the constitutional symptoms at the onset, the lack of at least mild degrees of fever at the onset of disease and the laboratory data, such as normal leukocyte count and rate of sedimentation of erythrocytes, suggests that a well-known bacterial agent is not the cause of the disease."[15] Beerman[4] has suggested that progressive systemic sclerosis may represent one of the diseases of adaptation.

HISTOPATHOLOGY

A swelling and separation of the collagen bundles of the skin and subcutaneous tissues (edema) is the first important microscopic finding. Chronic inflammatory infiltration around blood vessels and an obliterative sclerosing of the intima follow. The fine collagen fibers of the corium become hyalinized. Papillae are flattened, and the epidermis is thinned. The disappearance of skin appendages is noted occasionally. In the deeper layers fibrous connective tissue septa are also thickened. Involvement of the periodontal membrane has been reported.[11, 21] Peculiar changes have also been recognized in the heart, gastrointestinal tract, kidneys, esophagus, and lungs.[3-5, 9, 10, 17]

DISCUSSION

In 1944 Stafne and Austin[21] reported a characteristic widening of the periodontal space in acrosclerosis and scleroderma. Their review of dental roentgenograms in 127 cases revealed an incidence of 7.08 per cent. Their nine cases involved four male and five female patients. The thickening surrounded the entire tooth almost uniformly, thereby differing from periodontitis and traumatic occlusion. This thickening was at the expense of the lamina dura and not the teeth, which were firm in the sockets. In their cases the



A.

Fig. 2.—Case 1. Note characteristic thickening of periodontal membrane involving anterior and posterior teeth.
(Fig. 2, B and C on opposite page.)

posterior teeth were affected more often than anterior teeth. Rosenthal,[20] Krough,[12] and Gores,[11] have subsequently reported this finding. In each case a female patient was affected. The roentgenographic picture returned to normal in Krough's patient. This has not otherwise been reported. In one of the two cases to be discussed, a widening of the periodontal space (Fig. 2) was the only roentgenographic evidence of the disease, except for erosion of the ungual tufts of the distal phalanges of both hands and feet. In the other, although multiple roentgenographic findings were in evidence, the dental roentgenograms were not characteristic.

CASE REPORTS

CASE 1.—F. R., a 32-year-old white male dental technician, entered the Veterans Administration Hospital in Dearborn, Michigan, on Nov. 8, 1956, with a chief complaint of a 30-pound weight loss in two years. This was associated with a slight increase, of one year's

B.

C.

Fig. 2.—*B* and *C*. *(For legend, see opposite page.)*

duration, in fatigability and inability to close the fist tightly. No history of Raynaud's phenomenon or disease was obtained. The patient had not suffered from dysphagia. A few months before entering the hospital he had been successfully treated with cortisone tablets for occasional pain in the left knee.

Past history revealed that the patient had been wounded in the leg in 1945 and suffered a residual peroneal nerve paralysis.

Fig. 3.—Case 1. Note size of oral cavity and lack of wrinkles on face.

Physical examination revealed a moderately to poorly nourished white man with the typical masklike facies of scleroderma (Fig. 3). The lips were thin and tight, and an oral examination was accomplished with great difficulty. The gingiva was retracted from the lower anterior teeth by a scarlike band of tissue from the lower lip. The lower central incisors were loosened by this process. A moderate amount of resorption of the alveolar process had occurred and was believed secondary to the pressure of the tissue. There was a foot drop with prosthetic correction on the right. A definite tapering of the fingers, known as sclerodactylia, and inability to flex or extend the fingers were noted. The skin of the upper extremity, chest, abdomen, and fingers, as well as of the face, was taut and bound to the underlying subcutaneous tissues. There was an erythema of the chest, abdominal wall, and extremities but not of the face or the hands.

Dental roentgenograms revealed a thickening of the periodontal membrane at the expense of the lamina dura (Fig. 2). Subsequent roentgenograms on Aug. 6, 1957, revealed no significant changes from the November examination.

Roentgenograms of the hands and feet disclosed erosion of the ungual tufts of the distal phalanges compatible with a diagnosis of scleroderma. Fluoroscopy of the esophagus was essentially normal, as was the remainder of the physical examination. The laboratory data were within the limits of normal.

The patient was presented to a medical conference, at which time it was decided that he was not ill enough to warrant risk of steroid therapy. He was placed on chloroquine and vitamin E.

CASE 2.—J. G. (Fig. 4), a 26-year-old white man, was admitted to the Veterans Administration Hospital in Dearborn, Michigan, on Nov. 5, 1956, with a chief complaint of joint pain of eighteen months' duration and dysphagia of three to four weeks' duration. The patient stated that he had been well until January, 1952, when he noticed some swelling of the right knee. This acute episode subsided but recurred in Korea especially during activity or damp weather. In January, 1955, the patient noticed intermittent swelling and pain of both knees, associated with heat and redness. In April, 1955, inability to flex the fingers of his right hand was noted. The left side was involved four months later. Pain did not begin in the hands until the early spring of 1956, by which time the patient was unable to make a fist with either hand. Stiffness was also present in the ankles, elbows, and shoulders.

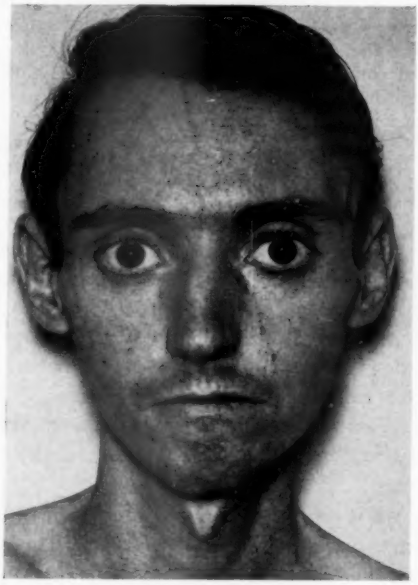


Fig. 4.—Case 2. Typical masklike facies with thin lips, lack of facial wrinkles, and "pinched" appearance of nose.

In August, 1956, an ulcer developed on the radical aspect of the right ankle following trauma. This did not respond to any therapy instituted by his private physician. Three to four weeks prior to hospitalization the patient noticed that food seemed to stop at the cervical portion of the esophagus, causing him to regurgitate or swallow with marked discomfort. The past history was otherwise noncontributory.

Physical examination revealed a slender, emaciated white man who was alert and cooperative. The skin over the face was shiny, thin, waxy, and tightly bound to the subcutaneous tissue. It could not be picked up between the fingers. The lips were pursed and oral examination was extremely difficult. There was definite narrowing of the oral orifice. Oral hygiene was poor. The patient admitted to personal neglect fostered by his inability to hold a toothbrush (Fig. 1). The remainder of the oral examination was essentially negative except for several supernumerary teeth.

There was flexion deformity of the interphalangeal joints and fusiform swelling of the distal phalangeal joints. The finger tips were cool. The ankles and knees were swollen and deformed, and the previously described ulcer was present on the right ankle. The skin over the hands was taut and parchmentlike in consistency (Fig. 1).

The laboratory data were within the limits of normal except for an erythrocyte sedimentation rate of 38. *Staphylococcus aureus*, coagulase negative, was cultured from the ulceration on the right ankle.

Roentgenograms revealed moderately diffuse fibrosis of both lung fields compatible with scleroderma. The terminal phalanges of the fingers, including the thumb, were missing on roentgenographic examination. An esophagram showed some dilatation of the upper esophagus and narrowing of the lower esophagus. Peristalsis in the upper esophagus was ineffective. This was compatible with scleroderma. A specimen of skin from the right wrist showed acrosclerosis. Dental roentgenograms revealed several supernumerary impacted as well as erupted teeth. The periodontal membrane appeared normal.

The patient was presented to the same medical conference as the patient in Case 1 because of the unusual occurrence of scleroderma in young men. It was felt that ACTH was not indicated, inasmuch as the patient's complaints were quite minimal despite the extensive findings. He was placed on para-aminobenzoic acid and chloroquine. The patient noticed immediate improvement in his ability to swallow solid foods, but it was not known whether this was due to the medication or was psychological.

CONCLUSION

The most common finding of dental significance in scleroderma is the rigidity and thinness of the lips and the difficulty encountered in retracting them. This rigidity may prevent adequate opening and interfere with speech and mastication, as in the cases described by Afonsky[1] and Rosenthal.[20] Extreme pressure may even cause ulceration of the mucosa by the teeth. The claim that the tongue, the soft palate, and the larynx are frequently involved[13, 18] does not seem to be substantiated, judging from the paucity of cases reported in the literature. Frank[8] and Truelove and Whyte,[24] however, have reported interesting cases in which stiffness of the tongue was a prominent feature.

If the oral mucosa is involved, it is pale and thin and tends to ulcerate. Buccal pigmentation is rare. Pseudoankylosis may be caused by restricted mouth opening, and the obliteration of the fornix may lead to poor oral hygiene and its subsequent sequelae. This can also occur, as it did in Case 2, when there is sufficient crippling of the hands to prevent toothbrushing. The ankylosis may result also from involvement of the muscles of mastication. Although there is no change in the vascular components, hemorrhage following simple tooth extraction may be severe.[14] This is believed to result from the inability of the vessels to contract. It may be difficult to secure intraoral roentgenograms. Once obtained, they may demonstrate the characteristic findings already discussed. Periodic examinations should be made to determine whether these changes are generally permanent or transitory. Tilting of the teeth and resorption of the alveolar ridge,[23] recession of the gingivae,[6] delayed tooth eruption,[13] and exfoliation of teeth[1] may result from extrinsic pressure.

Both of the patients discussed in this article manifested enough oral evidence of scleroderma to warrant referral to the medical service had they originally presented themselves to the dental clinic. One patient (Case 1) has returned to work as a dental technician and is checked periodically in the dermatology and dental clinics. The dental roentgenograms have not changed. The other patient (Case 2) could not be contacted.

SUMMARY

1. The various forms of progressive systemic sclerosis (scleroderma) have been described, and the associated oral and dental findings have been discussed.

2. Two cases have been presented to demonstrate the manifestations of the disease.

3. The characteristic dental roentgenographic finding of widening of the periodontal membrane was observed in one patient.

4. It is recommended that all patients with progressive systemic sclerosis receive periodic dental roentgenographic examination to determine whether the changes in the periodontal membrane are permanent or transitory.

5. The dental profession is again reminded of its obligation to detect systemic diseases when they present themselves in the oral cavity.

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PHARMACOLOGY AND THERAPEUTICS

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A CLINICAL APPRAISAL OF HYALURONIDASE IN DENTAL PROCEDURES

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SURGICAL diathermy (electrosurgery) is a valuable technique in dental practice, permitting many procedures that otherwise would be difficult and time consuming if performed in the office. One objection to the use of electrosurgery,[3] however, is the localization of heat at points distant from the actual site of surgery, namely, areas not affected by the usual local anesthetics. The search for a better anesthetic to improve surgical diathermic operations has led to the use of additives to the procaine solutions now in use.

Dental literature[2] indicates that the enzyme hyaluronidase* possesses peculiar penetrating and spreading properties. By depolymerizing hyaluronic acid, thought to be the major chemical component of the connective tissue "ground substance," hyaluronidase increases tissue permeability and opens pathways for freer diffusion and absorption of fluids.[1] This increases the speed of onset, depth, and area of local anesthetics. When hyaluronidase is mixed in solution at room temperature with procaine, it remains stable for two weeks. In view of such limited stability, it was considered advantageous to develop a simple technique for the routine use of hyaluronidase-lidocaine† solution in order that the same facility and economy would be possible as in the use of the usual Carpule. With this problem in mind, the present study was undertaken.

PROCEDURE

Two series of tests were organized, involving 1,000 procedures. One series, consisting of 100 gingival retractions preparatory to the taking of hydrocolloid impressions for inlays on posterior teeth, was studied to test the adequate spread of small quantities of hyaluronidase-lidocaine solution. The

*Hyaluronidase is available as Wydase (lyophilized or stabilized solution) from Wyeth Laboratories, Philadelphia, Pennsylvania. The U.S.P. unit is equivalent to the turbidity-reducing unit.

†Xylocaine hydrochloride, Astra Pharmaceutical Products, Inc., Worcester, Massachusetts.

second series, performed on fifty-five patients, involved preparation of gingival cavities on mandibular second premolars with a simple infiltration of the hyaluronidase-lidocaine mixture. A Coles Radiosurg machine was employed, with the hemostatic current set at 1.5. The Radiosurg point was moved through the interseptal space until the gingival margin of the cavity was clear of tissue.

Vim-Gabriel syringes (2 c.c. and 5 c.c. sizes), which permit aspiration prior to injection, were used for injection and mixing of the hyaluronidase-lidocaine solution. At the tip of the syringe is a short (½ inch long) needle-like metal fitting over which the hypodermic needle can be placed when the syringe is used for injection. This short projection was inserted through the rubber stopper of the 1 c.c. vial of hyaluronidase and the contents were conveyed to the 20 c.c. vial of lidocaine, thus mixing the two solutions. This method protects the needle from undue damage in preparation of the hyaluronidase-lidocaine solution and avoids contamination of the hyaluronidase vial. The needles, kept in sterile Petri dishes, were affixed to the metal fitting with sterile forceps, and the syringe was ready for injection of the first patient. To obtain positive pressure in the hyaluronidase-lidocaine solution and to avoid air bubbles when filling the syringe, air equal to the amount of solution to be withdrawn was injected into the vial. The syringes were kept in the warming compartment of an autoclave or incubator. Thus, the solution was brought to near body temperature on withdrawal from the vial into the warmed syringe.

The stock solution of hyaluronidase-lidocaine can be kept in a dark place ready for use. If, for any reason, the full 20 c.c. is not consumed within two weeks, however, the efficacy of lidocaine is in no way impaired and more hyaluronidase can be added to renew the strength of the solution.

The dose of the hyaluronidase-lidocaine solution (150 units of hyaluronidase to 20 c.c. of lidocaine 1:50,000) varied from 0.25 c.c. injected into the buccal gingivae to 0.75 c.c. infiltrated opposite the teeth.

RESULTS

In all cases there was a complete absence of pain or diathermic sensation. The patient appeared calm and without the tenseness that accompanies apprehension. It was soon found that only one-third to one-half the usual amount of solution produced anesthesia with enough spread to enable the performance of all electrosurgical operations without discomfort. As the solution was diffused palatally, anesthesia was complete. Thus, it was possible to carry out procedures that heretofore could not be performed without supplementary injections. In the preparation of gingival cavities the patients were relieved of discomfort and, hence, were less apprehensive. The period of anesthesia, although not prolonged by addition of hyaluronidase to the anesthetic, allowed ample time to complete all work. Little or no swelling accompanied the operations, and anesthesia was terminated without complaint from the patient.

SUMMARY

Observations were made on 1,000 procedures in which a hyaluronidase-lidocaine solution was employed for local anesthesia in dental surgery. Addition of hyaluronidase to lidocaine by the simple technique described provides an improved anesthesia which appears adequate for the majority of dental operations. Administration of this solution has proved useful in the preparation of cavities and in gingival retraction by electrosurgical methods and affords an economic advantage over the use of Carpules without hyaluronidase.

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1352 DAVID WHITNEY BLDG.

THE USE OF A COMBINATION OF NEOMYCIN, BACITRACIN, AND POLYMYXIN IN ENDODONTIA

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INTRODUCTION

In recent years the conservative management of infected root canals has been assisted by the topical use of chemotherapeutic agents, in particular various polyantibiotic combinations. While the clinical results appear to be most encouraging,[1, 3] it is still debatable whether such combinations are theoretically acceptable.[4, 6, 7] For example, an ideal combination of antibacterial drugs for endodontia is one which will be rapidly bactericidal against a wide range of microbial species, which will maintain its activity in situ for at least 48 hours, which readily diffuses into the dentinal tubules, which is nonirritant to periapical tissues, and the use of which does not carry the risk of sensitization. None of the currently used preparations meets all these requirements, although some, particularly the polyantibiotics, satisfy most of the major criteria used. The possibility that a combination of antibiotics could be selected to meet these criteria has now been investigated.

The present paper deals with the development of such a combination and the indications for its use in endodontia. In order to demonstrate specific attributes, special techniques were devised, some of which could form the basis for future testing of antibacterial combinations intended for root canal therapy. From the observations recorded here, a solution containing neomycin, bacitracin, polymyxin, a fungicide, 5-chloro-2-(*p*-diethylamino-ethoxyphenyl) benzthiazole tartrate (A.163, Crookes), and noradrenaline, hereafter referred to as ATF (antibiotic triple formula), is recommended as a chemotherapeutic combination more suited to root canal therapy than any hitherto described.

MATERIALS AND METHODS

1. *Cultures.*—All cultures used in this study have been those maintained in our Departmental culture collection.

2. *Antibiotics and Other Substances.*—The formulation under investigation consisted of the following substances: neomycin sulfate, 70 units per milligram; bacitracin, 70 units per milligram; polymyxin B sulfate, 7,500

From the School of Bacteriology and School of Dental Science, University of Melbourne.

units per microgram; and 5-chloro-2-(*p*-diethylamino-ethoxyphenyl) benzthiazole tartrate (A.163).* The actual composition will de discussed later in this paper. The following combinations were used in comparative investigations:

(a) PBSC (Grossman[3])—penicillin, bacitracin, streptomycin, sodium caprylate.

(b) PSCC (Bender and Seltzer[1])—penicillin, streptomycin, chloramphenicol, sodium caprylate.

(c) ACB (Sullivan and Jolly[7])—aminacrine, cetrimide, hydroxybenzoates.

(d) Camphorated *p*-chlorophenol.

(e) Formocresol (Buckley[2])—trieresol and formaldehyde.

(f) Beechwood creosote.

3. *Bactericidal Activity.*—There are a number of ways of carrying out a bactericidal test. The method developed in this study consisted in using small acrylic (Perspex) cylinders (approximately 1.5 by 1 cm.) in which a central canal, about the size of a root canal, had been drilled almost to the length of the cylinder (Fig. 1).

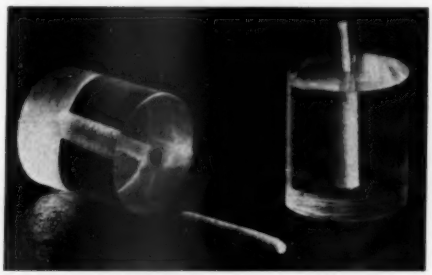


Fig. 1.—Acrylic buttons for bactericidal and fungicidal tests.

These "buttons" were sterilized by autoclaving at 115° C. (240° F.) for 15 minutes and the central canal inoculated with a fine loop of a 24-hour fluid culture of the test organism. When the contaminated canal was dry, it was filled with the antibacterial solution by means of a Pasteur pipette, and a dry sterile paper point was inserted into it. The paper point, which absorbed all the free fluid, was removed at various times and the acrylic button was cultured in 10 ml. of an appropriate fluid medium. The tubes were incubated at 37° C. and presence or absence of growth was noted over a suitable period of time depending on the organism concerned, that is, not less than 3 days and up to 3 weeks in the case of *Mycobacterium tuberculosis.* (Fig. 2.)

Each test was repeated several times on separate occasions and was controlled in the following manner:

a. Contaminated buttons were treated as above, using sterile saline in place of the antibiotic, and then cultured. This control showed that sterility was not due to the mechanical removal or natural die-away of the test organism.

*Kindly supplied by Crookes Laboratories, London.

b. Sterile buttons were treated as above with the antibiotic and placed into test tubes containing media previously inoculated with the test organism. These tubes were then incubated. This control showed that the carry-over of antibiotic was insufficient to exert bacteriostasis of the test organism.

This technique is a severe test since it measures complete (100 per cent) bactericidal activity—a single surviving viable unit would produce a positive culture. Also, it simulated the actual dental manipulation in root canal therapy and could be easily adapted to measure fungicidal activity of various fungicides and the effect of dilution, of blood and of serum on the rate of kill.

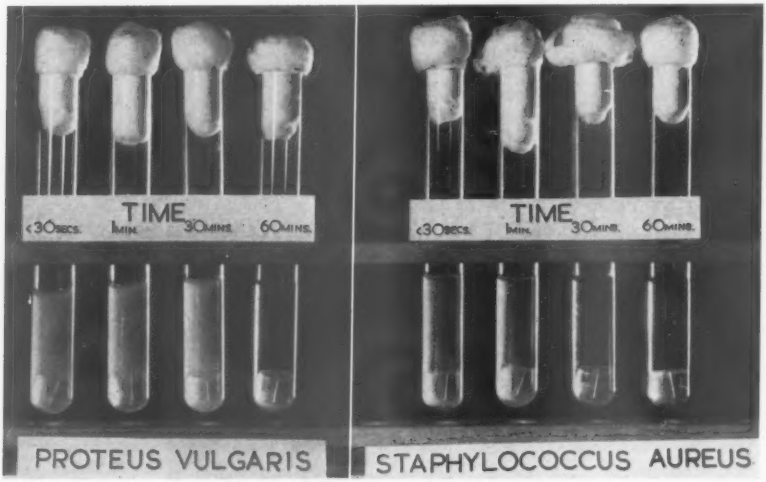


Fig. 2.—A typical result of a bactericidal test, showing sterilization of *Proteus vulgaris* (60 minutes) and *Staphylococcus aureus* (30 minutes).

4. *Effect of Blood and Serum on the Bactericidal Activity of ATF.*—This was tested by repeating the bactericidal tests with the following modifications: (a) dissolving ATF in 50 per cent blood-water; (b) suspending the test organisms in whole blood prior to contaminating the walls of the canal; (c) combining both (a) and (b).

5. *Effect of Necrotic Tissue on the Bactericidal Activity of ATF.*—Mandibular first molars with obviously necrotic pulps were extracted and the teeth were split vertically in a buccolingual plane. One half of each tooth was cultured aerobically in broth and the other half was immersed in ATF solution for 1, 12, or 24 hours at room temperature. The treated teeth were removed from the solution, washed repeatedly in sterile water, and then cultured in 100 ml. of broth at 37° C. for 3 days. If no growth occurred in the latter media, they were inoculated with *Staphylococcus aureus* in order to prove that the apparent sterility was not due to a carry-over of a bacteriostatic amount of ATF.

6. *Diffusion Through Dentinal Tubules.*—While most workers assume that antibiotics inserted into the root canal will diffuse freely into dentinal tubules, we did not feel this assumption was justified without experimental demonstration. The following method was developed to demonstrate diffusibility through the dentine.

Healthy human upper anterior teeth were obtained during full clearance prior to the insertion of dentures. The pulp was removed within 3 hours of extraction and the root canal cleaned with suitable instruments. The apical third of the root was closed with sticky wax and a dry paper point was inserted into the pulp canal. The test solution was then introduced by means of a Pasteur pipette so that the paper point became saturated in the solution. The coronal opening into the canal was sealed with sticky wax and the whole tooth was then coated by dipping repeatedly in melted beeswax. With one set of teeth, windows were cut through the wax down to primary cementum, with others the windows were continued through the primary cementum to dentine, and with another set the windows were extended to the pulp canal.

A tooth so prepared was placed, window up, in a Petri dish, and a drop of melted agar was placed in the window to fill the space and prevent air bubble formation. Agar was then poured into the dish until the tooth was just covered. When the agar solidified, the surface was seeded with the test organism to produce a lawn culture.

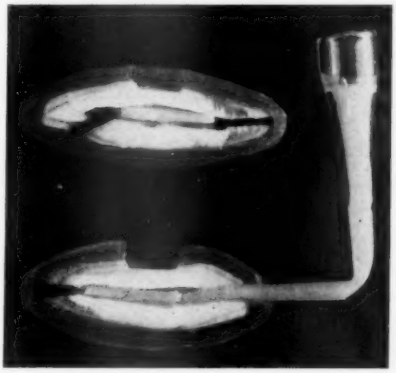


Fig. 3.—Sectional views demonstrating the preparation of recently extracted teeth to test for diffusion through the dentine. In the upper tooth a window has been cut down to the dentine immediately over a paper point lying in the root canal; the lower tooth has been similarly prepared, but a reservoir of antimicrobial is sealed into the canal.

Diffusion of the antibacterial into the medium was assessed by noting the appearance of a zone of inhibition in the lawn culture. Where a substance did not appear to diffuse—as shown by the failure of an area of inhibition to appear in the lawn—the experiment was repeated with the following modification. A reservoir in the form of a small glass funnel with an angled stem was fixed in continuity with the pulp canal and then filled with the antibacterial solution (Fig. 3). In this way an excess of solution was always available for

saturating the paper point and diffusing through the tooth structure into the agar. Thus, failure to inhibit growth under these conditions could be interpreted as a failure to diffuse through the dentinal tubules.

7. *Tissue Toxicity.*—The rabbit ear method described by Gronberg (1935), quoted by Wielding,[8] was used. In this, 0.1 ml. of the test solution is injected subcutaneously into the outer surface of the rabbit's ear, and degree of damage, as evidenced by hyperemia, edema, necrosis, and ulceration, is recorded over a period of 7 days.

Toxic action against tissue cells was determined by observing the effect of ATF and other preparations on the morphology (hemolysis and staining reactions) of the cellular elements of whole blood.

In addition, twenty-four human subjects were given intradermal injections of 0.1 ml. amounts of ATF, and others were treated with ATF by inserting into bone cavities, following curettage or apicectomy, a sterile cellulose sponge saturated with the solution.

8. *Stability In Vivo and In Vitro.*—It was considered important that any antibacterial preparation recommended for endodontia should retain at least 50 per cent of its original activity for 48 hours after insertion into the root canal. This time was selected as a minimum since patients may not receive treatment during week ends. The stability in vivo was determined by insertion of ATF into the prepared root canals of dogs and, later, in the root canal of a patient.

Under Nembutal anesthesia, upper and lower canines of the dog were opened and the pulps removed. The root canals were cleaned and broaches were pushed through into the periapical tissues to encourage hemorrhage. Each of the four teeth so treated was irrigated with ATF solution and paper points saturated with the antibiotic were inserted. The root canals were sealed with wax and the cavities filled with zinc phosphate cement. After 48 hours the paper points were removed (under anesthesia) and placed on agar media previously seeded with *Staph. aureus.* The degree of inhibition of growth around the paper points was compared with that obtained with paper points saturated with the antibiotic solution and kept for 48 hours at 37° C. in sealed tubes.

Stability in vitro was determined by retesting ATF solution at various times for activity against different species, using the disc inhibition technique.

RESULTS

The starting point in these investigations was a formula suggested by one of us (Rubbo[6]) which consisted of a combination of neomycin, bacitracin, and sodium caprylate, termed NBC. It soon became evident as the present work progressed that the combination was ineffective against *Pseudomonas pyocyanea,* that sodium caprylate was inactive against *Candida albicans* in concentrations which were nonirritant to tissues and was chemically incompatible with

neomycin. Thus, the formula was modified by including polymyxin B sulfate, deleting sodium caprylate, and substituting, after extensive investigation of a number of antifungal agents, 5-chloro-2-(p-diethylamino-ethoxyphenyl) benzthiazole tartrate (A.163, Crookes) as a fungicide. For convenience, the final formula is now referred to as ATF.

In making our choice of the ATF formulation we were concerned with the measurement of five criteria:

a. The mixture had to be rapidly bactericidal and fungicidal in the presence of blood or serum for a wide range of microbial species found in root canals and in the oral cavity.

b. The mixture had to diffuse readily through dentinal tubules.

c. The mixture had to be nontoxic to tissues and tissue cells.

d. The mixture had to withstand reasonable dilution (e.g., twofold) in situ without loss of effective bactericidal action.

e. The mixture had to be stable for at least 48 hours in the root canal.

Many combinations of neomycin, bacitracin, and polymyxin were studied and no useful purpose would be served in tabulating the protocols of these experiments. It is sufficient to point out that the ATF formula was not empirically selected and that the combination finally chosen was, according to our tests, best adapted to meet the five criteria set out above. The selection of a fungicide proved most difficult and will be dealt with separately. Noradrenaline was added to reduce dilution of ATF by hemorrhage into the root canal and to prevent its diffusion from the periapical tissues during the bactericidal period, which, in most instances, was less than one hour. Sorbitol has been included as an excipient to facilitate dispensing (see later) and was chosen because none of the organisms likely to be encountered in root canals can use this "sugar" as a fermentable substrate.

The proposed formulation of ATF consists of the following dissolved in 1 ml. sterile water, the final pH being 5.7.

Neomycin sulfate (70 units per mg.)	20 mg.
Bacitracin (70 units per mg.)	5 mg.
Polymyxin B sulfate (7,500 units per μg)	1 mg.
A.163 (Crookes Laboratories)	0.5 mg.
Noradrenaline	0.1 mg.
Sorbitol (as an excipient) to	100 mg.

1. *Bactericidal Activity of ATF.*—Twenty organisms were used to determine the bactericidal and fungicidal spectrum of ATF and the tests were repeated on a number of separate occasions. The number of organisms inoculated in the acrylic canals was determined for several species and found to be approximately 30,000 to 100,000 depending on the type of organism used. Table I summarizes the collected results. It will be seen that all species tested, except *Clostridium sporogenes* (sporing culture) and *C. albicans*, were killed in one hour and that most species were destroyed in 30 minutes. As indicated in Table

I, ATF is lethal to *C. albicans* after five hours' exposure and to *Cl. sporogenes* spores after 24 hours. The viridans streptococci, which, in our experience, are most commonly isolated from root canals, were instantly sterilized by contact with ATF. There can be little doubt from these findings that ATF is lethal for all bacteria and yeasts likely to be recovered from root canals.

TABLE I. BACTERICIDAL ACTIVITY OF ATF COMBINATION

ORGANISM	GROWTH AFTER CONTACT WITH ATF FOR				
	<30 SEC.	1 MIN.	15 MIN.	30 MIN.	60 MIN.
Gram-Positive Cocci					
Strep. salivarius (viridans strep.)	–	–	–	–	–
Strep. mitis (viridans strep.)	–	–	–	–	–
Strep. pneumonia (Type III)	+	–	–	–	–
Strep. pyogenes	+	+	–	–	–
Strep. faecalis	+	+	+	–	–
Staph. aureus (penicillin sens.)	+	+	+	–	–
Staph. aureus (penicillin res.)	+	+	+	–	–
Gram-Negative Coccus					
N. catarrhalis	–	–	–	–	–
Gram-Positive Bacilli					
B. subtilis (sporing)	+	+	–	–	–
Lacto. acidophilus	+	–	–	–	–
C. diphtheriae mitis	+	+	–	–	–
Cl. sporogenes (sporing)	+	+	+	–	– (– at 24 hr.)
Myco. tuberculosis (human)	+	+	–	–	–
Gram-Negative Bacilli					
E. coli (Type I)	+	+	+	–	–
Ps. pyocyanea	+	+	+	–	–
Pr. vulgaris	+	+	+	+	–
Kl. pneumoniae	+	+	+	–	–
Yeasts					
Sacch. cerevisiae	+	+	+	+	–
C. albicans	+	+	+	+	+ (– at 5 hr.)
C. stellatoides	+	+	+	+	+ (– at 5 hr.)

+ signifies growth; – no growth, after 3 days' incubation at 37° C. (*Candida* sp. 7 days' incubation at 28° C., *Myco. tuberculosis* up to 21 days' incubation at 37° C.)

2. *Fungicidal Activity of ATF.*—*C. albicans* is not an infrequent cause of cutaneous and mucous membrane infections and has been recovered with a high degree of frequency (17-40 per cent) in root canal cultures.[4] Although the role of *C. albicans* has never been clearly defined in root canal infection, it is evident that the inclusion of a fungicide in an endodontic preparation is desirable.

In our experience, this organism is particularly difficult to kill and the ideal fungicide suitable for endodontia has yet to be developed. Grossman[3] and Bender and Seltzer[1] have recommended sodium caprylate; Sullivan and Jolly[7] have used hydroxybenzoates, and Holst[4] has suggested phenyl mercuriacetate. We have tested these and other fungicides according to the technique described above, and the results are listed in Table II.

TABLE II. ACTIVITY OF VARIOUS FUNGICIDES USED IN ENDODONTIA
Organism: *Candida albicans*

SUBSTANCE	CONCENTRATION IN WATER		TIME REQUIRED TO KILL*
	PER CENT	MG./ML.	
Nystatin (Mycostatin, Squibb)	0.2	2	1 hour
	0.1	1	3 hours
	0.05	0.5	5 hours
A.163, Crookes (See text)	0.2	2	2 hours
	0.1	1	3 hours
	0.05	0.5	5 hours
Sodium caprylate	20	200	2 hours
Cetrimide	0.2	2	2 hours
Merthiolate	0.2	2	5 hours
Zinc undecylenate	10	100	>24 hours
Methyl *p*-hydroxybenzoate	0.4	4	>24 hours
Propyl *p*-hydroxybenzoate	0.1	1	>24 hours

*For technique, see text.

It is evident that nystatin and A.163 were the most active of the fungicides tested in that they were capable of killing *C. albicans* rapidly in low concentration. The hydroxybenzoates in the concentration recommended by Sullivan and Jolly were without effect, as was zinc undecylenate. Of the two fungicides of choice, A.163 was preferred to nystatin because it was more stable and showed no evidence of skin sensitization, as did nystatin, on intradermal injection. Our recommended concentration of 0.05 per cent (0.5 mg. per milliliter) killed *C. albicans* in five hours but hemolyzed red cells after twenty-four hours.

3. *Effect of Blood on Bactericidal Activity of ATF.*—It is obvious that the demonstration of the sterilizing action of ATF in the absence of blood could not be regarded as an index of its potential activity in vivo. Accordingly, the sterilization of some important species was re-examined in the presence of Group O human blood by the method previously described.

TABLE III. EFFECT OF BLOOD ON THE BACTERICIDAL ACTIVITY OF ATF

ORGANISM	GROWTH AFTER CONTACT WITH ATF					
	IN DISTILLED WATER			IN WHOLE BLOOD		
	15 MIN.	60 MIN.	5 HR.	15 MIN.	60 MIN.	5 HR.
Strep. salivarius	–	–	–	–	–	–
Strep. pyogenes	–	–	–	–	–	–
Staph. aureus	+	–	–	+	–	–
E. coli	+	–	–	+	–	–
C. albicans	+	+	–	+	+	–

The results are listed in Table III. They clearly indicate that there is no measurable reduction in bactericidal activity in the presence of blood. Serum, as would be expected, also did not affect the rate of kill. On this evidence, it can be concluded that postoperative serosanguineous oozing into the root canal will not affect the activity of ATF other than by mechanical dilution.

4. *Effect of Dilution on Bactericidal Activity of ATF.*—The degree of dilution which occurs in vivo cannot be accurately predicted and will vary from case to case depending on operative trauma, vascularity of the periapical tissues, etc. However, it was thought desirable to determine the extent to which ATF solution could be diluted and still exhibit bactericidal activity. Thus, dilutions of ATF solution were made, as indicated in Table IV, and the bactericidal and fungicidal activity of each was determined.

TABLE IV. EFFECT OF DILUTION ON THE BACTERICIDAL ACTIVITY OF ATF

ORGANISM	GROWTH AFTER CONTACT FOR 60 MIN. WITH ATF			
	UNDILUTED	TWOFOLD	FOURFOLD	EIGHTFOLD
Strep. salivarius	–	–	–	+
Strep. pyogenes	–	–	–	+
Staph. aureus	–	–	+	+
E. coli	–	–	+	+
Ps. pyocyanea	–	–	+	+
C. albicans (8 hours' contact with ATF)	–	–	+	+

It will be seen that the antimicrobial action of ATF is effectively maintained up to a twofold dilution for all organisms tested, but beyond this the bactericidal action, except for streptococci, is uncertain. However, bacteriostasis, not shown in Table IV, still persists up to an eightfold dilution of the solution. Clinically, these findings indicate that the sterilizing activity of ATF could be reduced by excessive bleeding but the bacteriostatic action would remain unaffected. This dilution effect can be partially counteracted by the presence of 1.10,000 noradrenaline in the ATF. This is well illustrated in Fig. 5, which shows that the residual antibacterial activity of a paper point from root canal is higher when saturated with ATF containing noradrenaline than one with ATF alone.

5. *Effect of Necrotic Tissue on Bactericidal Activity of ATF.*—Using the technique described above, we obtained no evidence to indicate that the presence of necrotic tissue affected the bactericidal activity of ATF.

6. *Diffusion Through Dentinal Tubules.*—It is generally agreed that since bacteria may spread from the root canal into the dentinal tubules and accessory canals, the effectiveness of topical chemotherapy is, in part, determined by the ability of the preparation to diffuse freely into these structures. Grossman[3] and later Holst[4] suggested the inclusion of silicone and Tween 60, respectively, to polyantibiotic preparations to facilitate their diffusion from root canals.

The technique of demonstrating diffusion has been described in an earlier section of this paper and a result, typical of many, is illustrated in Fig. 4. It will be noted that ATF will readily diffuse through the tubules and that the addition of 0.5 per cent Tween 80 increases, but not markedly, the amount of drug diffusing.

When these zones of inhibition of growth are compared, it is evident that the greatest amount of antibiotic is liberated through "windows" communicating

with the root canal. However, such differences as those observed, and shown in Fig. 4, are a measure of the amount of antibiotic escaping from the tooth and not a measure of the concentration gradient in the tooth structure.

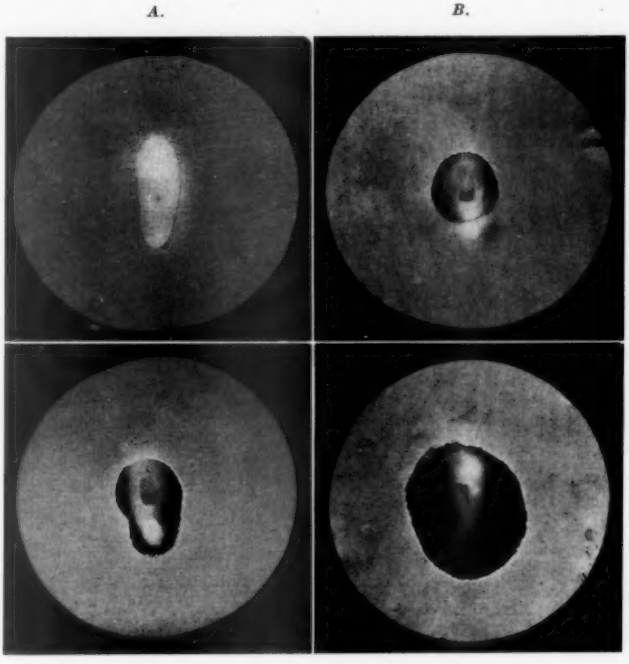


Fig. 4.—Lawn cultures of *Staphylococcus aureus* showing zones of inhibition of growth due to diffusion of ATF through windows cut to different levels of the tooth structure. *A*, Window cut through wax to cementum—no diffusion of ATF; *B*, window cut through both wax and cementum to dentine—diffusion, 11 mm. zone of inhibition; *C*, as for *B*, but with added Tween 80 (1:1,000)—diffusion, 12 mm. zone of inhibition; *D*, window communicating with root canal—diffusion, 21 mm. zone of inhibition.

Using the same technique we were unable to demonstrate any diffusion of Grossman's antibiotic paste, camphorated *p*-chlorophenol, beechwood creosote, or aminacrine-cetrimide (ACB above) through dentine. Modifying the technique (Fig. 3) by using a reservoir of the antimicrobial solution did not result in any effective diffusion through the tooth structure. These findings, summarized in Table V, underline the danger of assuming, without experiment, that antibacterial drugs will diffuse freely into the dentinal tubules.

TABLE V. DIFFUSION OF ANTIMICROBIAL PREPARATIONS THROUGH DENTINAL TUBULES
(See text for technical details)

PREPARATION	DIFFUSIBILITY THROUGH DENTINAL TUBULES
ATF	Positive
Grossman's PBSC (silicone paste)	Negative
Grossman's PBSC (aqueous solution)	Positive
ACB (Sullivan and Jolly)	Negative
Camphorated *p*-chlorophenol	Negative
Creosote (beechwood)	Negative

7. *Tissue Toxicity of ATF.*—This was tested by subcutaneous injection of ATF into the semitransparent tissues of the rabbit's ear, and has been used to determine the optimal and permissible concentration of ATF constituents. Many variations of ATF were used in these experiments and no useful purpose would be served by reporting these in full. However, in Table VI the limit of tissue tolerance to ATF is given. It will be seen that when ATF is used in the concentration recommended, no evidence of tissue irritation or damage is detectable (see also Fig. 6) and that a twofold increase in concentration is practically innocuous. Concentrations above this exhibit evidence of chemical irritation.

TABLE VI. TISSUE TOXICITY OF VARIOUS STRENGTHS OF ATF
(Subcutaneous injection of 0.1 ml. into rabbit's ear)

CONCENTRATION OF ATF	DEGREE OF REACTION AFTER		
	1 DAY	3 DAYS	7 DAYS
Recommended concentration	No reaction	No reaction	No reaction
Twice recommended concentration	No reaction	Slight erythema	No reaction
Four times recommended concentration	Slight erythema	Slight erythema; edema (5 mm.)	Slight erythema; no edema

Intradermal injection of 0.1 ml. of ATF into twenty-four human beings showed no evidence of serious tissue reaction. Within one hour of the injection a central raised area of blanching about 15 to 20 mm. in diameter was observed, and this was surrounded by diffuse erythematous flare of 20 to 40 mm. These reactions disappeared in four hours and at 24 hours there was no evidence of hyperemia or edema. When ATF was inserted into the periapical tissues on cellulose sponges, no toxic reactions were detectable clinically. These results indicate that ATF is devoid of significant tissue toxicity.

8. *Stability In Vivo and In Vitro.*—As indicated earlier, in vivo stability was studied in the dog by exposing the root canals of the four canine teeth, removing the pulps and inserting paper points saturated with ATF. After 48 hours the paper points were removed and placed on media previously inoculated with *Staph. aureus.* Control paper points saturated with antibiotic

TABLE VII. STABILITY OF ATF AT ROOM TEMPERATURE (15° C.)

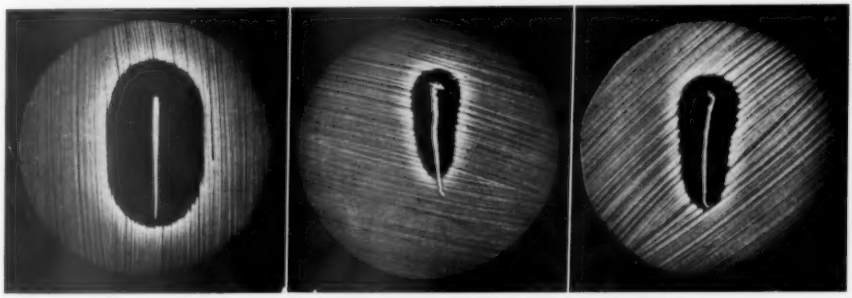
ORGANISM	ZONES OF INHIBITION IN MM. (1 CM. PAPER DISC) AFTER STANDING FOR			
	0 HR.	24 HR.	72 HR.	7 DAYS
Staph. aureus	27	28	28	28
Ps. pyocyanea	23	23	24	24
E. coli	27	27	26	25
C. albicans	14	14	14	15

were kept at 37° C. in sealed tubes for 48 hours and then transferred to the culture medium. Five dogs were subjected to the operation and the results were similar. A typical result is shown in Fig. 5, from which it can be calculated that almost 70 per cent of the original antibacterial activity is retained during

the 48-hour period in the tooth with noradrenaline, compared with 45 per cent without noradrenaline. For example, average zones of inhibition, measured at the widest section, were as follows:

ATF in vitro (control)	22 mm. zone of inhibition
ATF in vivo with noradrenaline	15 mm.
ATF in vivo without noradrenaline	10 mm.

These results were most encouraging, particularly as the degree of vascularity (and endodontic hemorrhage) is much more marked in the dog's tooth than in the human. Further, in one experiment, paper points were removed five days after insertion in pulp canals and these showed little reduction in antibacterial activity over this period. In patients receiving treatment there was no appreciable loss in activity of ATF even after remaining in teeth for seven days.



A. *B.* *C.*

Fig. 5.—Zones of inhibition of *Staphylococcus aureus* by ATF-saturated paper points treated in different ways: *A*, paper point kept at 37°C. for 72 hours (control); *B*, paper point removed from root canal of dog after 72 hours; *C*, as for *B*, but with addition of noradrenaline (1:10,000).

In vitro stability of ATF was also studied by assaying the solution after standing for various times up to 7 days at room temperature (15° C.). The results (Table VII) show that the antimicrobial activity of ATF is well maintained for at least 7 days at room temperature. However, for reasons given later, we recommend using freshly prepared solutions in clinical practice.

9. *Comparison of ATF With Other Antimicrobial Drugs Used in Endodontia.*—Many different types of antimicrobials have been used in endodontia, ranging from chemical disinfectants to polyantibiotics. Their value in endodontia may be assessed on theoretical, experimental, and clinical evidence. We shall confine the scope of this enquiry to an experimental comparison of the bactericidal activity and tissue toxicity of some of the more common substances and combinations in endodontia.

Bactericidal activity: A comparison of the bactericidal activities of some preparations is listed in Table VIII. It will be seen that formocresol and beechwood creosote are undoubtedly the most active bactericides of those tested

TABLE VIII. COMPARISON OF BACTERICIDAL ACTIVITY OF ATF WITH OTHER ANTIMICROBIAL PREPARATIONS USED IN ENDODONTIA

ORGANISM	ANTIMICROBIAL PREPARATION																							
	ATF				PBSC				PSCC				ACB				CAMPHORATED *p*-CHLOROPHENOL				FORMOCRESOL				CREOSOTE (BEECHWOOD)			
	30′	60′	24	HR.	30′	60′	24	HR.	30′	60′	24	HR.	30′	60′	24	HR.	30′	60′	24	HR.	30′	60′	24	HR.	30′	60′	24	HR.
Strep. salivarius	–	–	–		–	–	–		–	–	–		–	–	–		–	–	–		–	–	–		–	–	–	
Strep. faecalis	+	–	–		–	–	–		+	+	–		–	–	–		+	–	–		–	–	–		–	–	–	
Strep. pyogenes	–	–	–		–	–	–		–	–	–		–	–	–		+	+	–		–	–	–		–	–	–	
Staph. aureus	–	–	–		–	–	–		–	–	–		+	+	–		+	+	–		–	–	–		–	–	–	
E. coli	–	–	–		–	–	·		–	–	–		+	+	–		+	+	–		–	–	–		–	–	–	
Pr. vulgaris	+	–	–		–	–	–		–	–	–		+	+	–		+	–	–		–	–	–		–	–	–	
Ps. pyocyanea	+	–	–		+	–	–		–	–	–		–	–	–		+	–	–		–	–	–		–	–	–	
Kl. pneumoniae	–	–	–		+	+	–		–	–	–		+	+	–		–	–	–		–	–	–		–	–	–	
C. albicans	+	+	–		+	+	–		+	+	–		+	+	–		–	–	–		–	–	–		–	–	–	

+ signifies growth after contact with preparation; – signifies no growth (i.e., sterilization).

Formulae: ATF = neomycin, bacitracin, polymyxin, A.163.
PBSC = penicillin, bacitracin, streptomycin, sodium caprylate (Grossman[3]).
PSCC = penicillin, streptomycin, chloramphenicol, sodium caprylate (Bender and Seltzer[1]).
ACB = aminacrine, cetrimide, hydroxybenzoates (Sullivan and Jolly[7]).

(formocresol sterilizes instantaneously) and that ATF appears to be the next most active preparation when speed of disinfection is considered. However, since all showed pronounced and similar bactericidal action against a wide range of microbial species, no distinction between these preparations can be drawn.

Tissue toxicity: More importantly, the tissue toxicity tests clearly separated the preparations into two broad groups—the first causing gross tissue necrosis and the second producing no reaction. These results are shown in Table IX and illustrated in Fig. 6. The necrotizing group consists of camphorated *p*-chlorophenol, formocresol, oxpara liquid, and beechwood creosote, while ATF and the aminacrine-cetrimide combination fall into the second nontoxic group.

TABLE IX. COMPARISON OF TISSUE TOXICITY OF ATF AND OTHER ANTIMICROBIAL PREPARATIONS USED IN ENDODONTIA

PREPARATION	REACTION FOLLOWING SUBCUTANEOUS INJECTION OF 0.1 ML. INTO RABBIT'S EAR		
	AFTER 24 HOURS	AFTER 3 DAYS	AFTER 7 DAYS
ATF	No reaction	No reaction	No reaction
ACB (Sullivan and Jolly)	No reaction	No reaction	No reaction
Camphorated *p*-chlorophenol	Marked hemorrhagic necrosis	Same as for 24 hr.	Ulceration of necrotic area not involving full thickness of ear
Creosote (beechwood)	Parchmentlike area of necrosis with no inflammatory flare	Same as for 24 hr.	Parchment necrosis involving full thickness of ear
Formocresol	Parchmentlike area of necrosis surrounded by inflammatory flare	Same as for 24 hr.	Parchment necrosis involving full thickness of ear
Oxpara liquid	Parchmentlike area of necrosis with slight inflammatory flare	Same as for 24 hr., inflammatory flare more extensive	Parchment necrosis involving full thickness of ear

The severely damaging effect exhibited by the necrotizing materials is clearly shown in Fig. 6. It is evident that these substances, in spite of their marked bactericidal properties, have no place in topical chemotherapy and in endodontia. In contrast, ATF and ACB provoked no tissue reaction.

In addition, we examined the effect of these preparations on citrated whole blood (human) and a saline suspension of guinea pig leukocytes. As anticipated, camphorated *p*-chlorophenol, formocresol, and creosote caused an instantaneous denaturation and coagulation of blood and complete disintegration of leukocytes. The ACB formula of Sullivan and Jolly hemolyzed the blood (this was shown to be due to the cetrimide) but had no effect on the morphology of the white cells (Leishman stain). ATF was also without effect on white cells and only slowly hemolyzed red cells after twenty-four hours of exposure.

10. *Pharmaceutical Aspects of Dispensing ATF.*—Some attention was given in the present study to the method of dispensing ATF in a form which could be conveniently used in the dental surgery without risk of repeated contamination or loss of antimicrobial activity. As a result it is now recommended that ATF be dispensed as a sterile powder in 100 mg. amounts (see formula)

Fig. 6.—Reaction at 7 days to subcutaneous injection of 0.1 ml. of (*A*) formocresol—parchmentlike area of necrosis; (*B*) camphorated *p*-chlorophenol—necrosis and ulceration; (*C*) beechwood creosote—necrosis and ulceration; (*D*) oxpara liquid—parchmentlike area of necrosis; (*E*) ATF—no reaction; (*F*) ACB—no reaction.

in sterile, rubber-sealed vials, together with separate ampules containing 1 ml. of sterile distilled water. From these ingredients 1 ml. of ATF solution sufficient for a single treatment can be easily prepared with a sterile syringe and needle. The ATF solution should be used immediately for irrigating the canal, infiltrating the periapical tissues, and moistening paper points, as indicated later. In this way there would be no risk of using a contaminated preparation, as exists with other formulations. In hospital dental clinics, bulk ATF solutions could be prepared provided they are refrigerated and used aseptically within 7 days of dispensing.

DISCUSSION

The object of the present investigation has been to examine the potential chemotherapeutic value of a combination of neomycin, bacitracin, polymyxin, and 5-chloro-2-(*p*-diethylamino-ethoxyphenyl) benzthiazole tartrate (A.163, Crookes) in endodontia. From the observations reported here it would appear that such combination, referred to in the present paper as ATF, has a place in the management of infections of the root canal and surrounding structures.

Two distinct problems are involved in the chemotherapy of endodontic infection. There is the problem, first of the nature and location of the infecting organisms, and, second of the degree and extent of the infectious process. Theoretically, any of the oral flora may be found in root canals and periradicular sites, so that any antimicrobial preparation suitable for endodontia must have an exceedingly wide range of activity. The extent of the infectious process may range from surface contamination of a vital pulp to necrosis with infection extending into the periapical tissues. Chemotherapeutically, these extreme stages will require different treatment, but whatever chemotherapy is instituted it is obvious that chemical damage of the periapical tissues by topical chemotherapy must be avoided. Thus, the basic requirement of any chemotherapeutic preparation for the treatment of a root canal infection, however localized that infection may be, is a broad antimicrobial spectrum which is bactericidal at a concentration devoid of toxic effect on cellular and vascular mechanisms of defense and healing.

The question may now be asked as to whether ATF and other antimicrobial preparations recommended in endodontia meet this demand.

ATF is rapidly bactericidal and fungicidal toward a very wide range of microbial species likely to be found in root canals (Table I). It is not affected by blood (Table III), serum, or necrotic tissue and will stand up at least to twofold dilution without significant loss of bactericidal action (Table IV). It has been shown to penetrate effectively the dentine of freshly extracted teeth (Fig. 4), and in vivo it retains activity for several days (Fig. 5). Finally, it is nonirritant to tissues (Tables VI and IX and Fig. 6) and may be used to irrigate the root canal and infiltrate the periapical tissues. It can be concluded that ATF appears to be eminently suitable as a chemotherapeutic agent for endodontia.

The comparative studies reported here showed that all formulations used in endodontia possess good bactericidal activity (Table VIII) but that camphorated *p*-chlorophenol, formocresol, oxpara liquid, and creosote cause marked necrosis of tissue (Fig. 6). For this reason their use in endodontic therapy is contraindicated in spite of their marked bactericidal action. The chemical damage induced by these protoplasmic poisons is probably just as responsible for the repeated treatment of root canal infection as the presence of the infectious agent they are intended to destroy.

In regard to the various polyantibiotics now recommended, several objections can be raised to their use and these were discussed in an earlier paper,[6] in particular the risk of sensitization. It has also been shown here (Table V) that the silicone paste recommended by Grossman will not diffuse through dentinal tubules when inserted into the root canal. It is also unlikely that any antibacterial paste will diffuse through the foramen into the periapical tissues. The formulation of Sullivan and Jolly[7] also fails to diffuse through dentine and is markedly hemolytic.

One of the main difficulties in the present study has been to find a suitable fungicidal agent lethal to *C. albicans* but at the same time nontoxic to tissues. The best fungicides of those tested here were 5-chloro-2-(*p*-diethylaminoethoxyphenyl) benzthiazole tartrate (A.163, Crookes) and nystatin. Neither of these showed tissue toxicity in the rabbit ear test at fungicidal concentrations, but nystatin induced delayed skin sensitization in some individuals following intradermal injection. Accordingly, A.163 was chosen as the fungicide of choice.

It might be appropriate at this stage to discuss the management of root canal infections and to indicate the role of ATF in treatment. For the purpose of the present discussion, root canal infections have been classified into two main categories, those which are uncomplicated and those with complications:

Uncomplicated Infections.—This group includes the exposed vital pulps and those cases with necrotic pulps in which there is no clinical evidence of spread of infection beyond the root canal. Roentgenographically, there is an intact lamina dura and no evidence of periapical bone resorption.

Complicated Infections.—This group includes the nonvital pulps with clinical or roentgenographical evidence, or both, of periapical pathology. This group is subdivided into: (a) those cases showing diffuse periapical radiolucency, and (b) those cases showing a well-defined localized radiolucency.

The management of uncomplicated cases is as follows: The rubber dam is applied, tooth and surrounding tissues are swabbed with isopropyl tincture of iodine.[5] The pulp is removed and mechanical preparation of the canal is now undertaken. Hydrogen peroxide (10 vol.) diluted 1:10 in water may be used for irrigation during this stage. It is not important to enlarge the apical foramen for periapical infiltration in these uncomplicated cases. Irrigation of the canal with the aid of a syringe is carried out, using approximately 1 ml. of ATF solution. A dry paper point is inserted into the canal and then saturated with ATF. The canal is sealed with a pellet of wax, and a hot instrument

and zinc phosphate cement is used to cover the wax. The canal is filled at the next visit, using gutta percha moistened in chloroform only. Irritant cements must be avoided.

When dealing with complicated cases, the advisability of retaining the tooth must be considered on both dental and medical grounds. If it is decided to attempt to retain the tooth, it might be advisable to use a four-day chemotherapeutic cover, particularly with those cases showing diffuse radiolucency in the periapical tissue. The cover should have a wide spectrum of activity (e.g., penicillin and streptomycin or tetracycline).

If a fluctuant swelling is present over the region of the tooth root, this is incised to establish drainage. The pulp canal is opened and débrided as thoroughly as possible and an attempt is made to penetrate through the apex. For this maneuver, the tooth may have to be steadied with a splint of impression compound. The canal and periapical tissues are irrigated with ATF solution and a dry paper point is inserted and then saturated with ATF. The pulp canal is sealed with a pellet of beeswax. In these cases an additional cement seal is not recommended at the first sitting, in order to allow the patient to remove the wax with the aid of a pin, should discomfort arise. So far this has not been necessary, even in the treatment of acute cases.

Roentgenographic examination will allow these cases with complications to be divided into two subgroups, as indicated above.

Those with diffuse radiolucency are treated conservatively by repeated irrigation and periapical infiltration with ATF solution until there is no visible red-brown or yellowish discharge on the paper point. In addition, systemic chemotherapy should always be considered in these cases. In our experience, such cases have responded in 2 to 5 sittings, depending on the severity of the infection.

In the second subgroup of cases there is a well-defined localized radiolucency in relation to the apex of the root which is regarded as an indication of an underlying chronic pathological process. Such cases, however, may present with an acute exacerbation and it is impossible to assess accurately the pathological state of this lesion on the basis of roentgenographic appearance alone. While it is conceded that such localized lesions do at times respond to nonsurgical measures, the treatment of these cases by apicectomy or periapical curettage appears to be more in keeping with pathological and surgical principles. In this group simultaneous root filling and apicectomy combined with the use of ATF as indicated above has given good results.

In conclusion, the clinical results so far obtained with ATF lend strong support to the experimental work described here and we hope to present a fuller report of our clinical findings later.

SUMMARY

1. Experimental evidence is presented to support the claim that an antibiotic formula, ATF, consisting of an aqueous solution of neomycin, bacitracin,

polymyxin, and 5-chloro-2-(*p*-diethylamino-ethoxyphenyl) benzthiazole tartrate (A.163, Crookes) with 1:10,000 noradrenaline is eminently suitable for topical chemotherapy in endodontia.

2. The advantages of ATF over currently used formulations are presented.

3. The clinical application of ATF in treatment of infections involving the root canal and surrounding structures is briefly discussed.

We are indebted to the following organizations for the generous supply of the antimicrobial agents: Andrews Laboratories Pty. Ltd. (Sydney) for neomycin, bacitracin, polymyxin B sulfate and sodium caprylate; Crookes Laboratories Limited (London) for 5-chloro-2-(*p*-diethylamino-ethoxyphenyl) benzthiazole tartrate; and E. R. Squibb & Sons (London) for Mycostatin brand of nystatin.

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ORAL PATHOLOGY

OXYPHILIC GRANULAR CELL ADENOMA (ONCOCYTOMA)

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OXYPHILIC granular cell adenoma is an extremely uncommon neoplasm of salivary glands. As the name implies, the tumor is of glandular origin and is composed of large epithelial cells containing oxyphilic granular cytoplasm. These cells were designated "onkocytes" by Hamperl[1] in 1931. They were first described by Schaffer,[2] in 1897, as "swollen granular cells." They usually possess distinct cell boundaries, their nuclei being small and vesicular but at times pyknotic and irregular. The occasional pyknotic nature of the nuclei induced Zimmermann,[3] in 1927, to name these cells "pyknocytes." Schaffer[2] found these cells in the ducts and acini of the glands of the tongue, uvula, pharynx, and esophagus. Zimmermann[3] observed them in the sublingual glands as well as in the glands of the floor of the mouth and of the trachea. Hamperl,[4, 5] in an extensive study of these cells, found them to be present in the major and minor salivary glands, thyroid and parathyroids, hypophysis (both anterior and posterior lobes), testicles, fallopian tubes, pancreas, liver, stomach, and the glands of the pharynx, trachea, and esophagus. He studied the uvular, sublingual, and submaxillary glands of eighty-five persons and found these cells more frequently in persons between 50 and 70 years of age and almost always in those over 70. Sometimes these cells were aggregated in hyperplastic nodules. Skorpil,[6] examining 100 salivary glands, found oncocytes in five of them as hyperplastic nodules. Stout[7] noted oncocytes in the acini and ducts of the mucous glands in the bronchi. Stump[8] found these cells in the ducts of the "mucous glands of the vocal cords and the tonsillar capsule." Meza-Chavez[9] studied 100 parotid glands obtained from fifty-one persons ranging in age from 49 to 75 years. These cells were present in both parotid glands in eight persons and in a ninth person from whom only one parotid was removed. In four of his nine cases the oxyphilic granular cells

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were forming areas of "hyperplasia of the adenomatous type." These cells were distributed equally in both sexes and were found in ducts or acini, or both. In 1953 Bauer and Bauer[10] found these cells in the form of solid clusters and hyperplastic nodules in association with ducts in only eight of fifty-seven salivary glands in an age range of 61 to 92 years. They could not demonstrate these cells in the acinar epithelium. There are different views concerning the origin of these cells. Schaffer attributed their origin to the process of degeneration of the parenchymatous cells. Pischinger[11] interpreted them as undifferentiated cells which, by amitotic division and further development, were substituted for the destroyed glandular elements. Zimmermann considered them anomalous cells, since they are not present in all persons. He also suggested that they may be the result of an abnormal form of cellular transformation. Hamperl believed that "the oncocytes are the products of a transformation of the parenchymal cells of the respective organs and represent a peculiar degeneration." This transformation, in his opinion, appeared irreversible due to the fact that the number of oncocytes increased with age. Bauer and Bauer believed that oxyphilic granular cells resulted from cytoplasmic alteration of the ductal cells. The cause and the mode of the cytoplasmic changes are still obscure. Since these cells are found mostly in older persons, aging may be a responsible factor in inducing such changes in the cytoplasm of the parenchymatous cells.

Clinically, oxyphilic granular cell adenoma is indistinguishable from mixed tumor. It is usually a firm, well-demarcated, smooth or nodular swelling which is freely movable and is attached to neither the overlying skin nor the underlying deep structures. The swelling is not usually associated with pain, discomfort, or weakness of the facial nerve except in cases where the tumor is intimately attached to the facial nerve, as in the case described by Berkheiser and Clough.[12] Grossly, the tumor is a round or oval, well-encapsulated solid mass which is whitish to grayish red in color. The cut surface may be of various colors and is divided into lobules by a thin strand of fibrous connective tissue. Microscopically, the neoplasm is composed of large cells of different sizes and shapes, sometimes similar in appearance to hepatic or adrenal cortical cells (Fig. 1). The cells contain abundant cytoplasm which is granular in appearance. The granules are small and uniform and stain intensely with eosin, purplish blue with Mallory's aniline blue, and reddish with Masson's trichrome stain. The nuclei are small, round or oval, and may be stained with uniform intensity or be vesicular with one or more prominent nucleoli (Fig. 2). Sometimes the cells may be multinucleated, or the nucleus may be multilobed. The cells may be arranged in columns, cords, or whorls separated by connective tissue stroma which may be infiltrated with neoplastic cells. Occasionally, the cells may be arranged in tubular or acinar fashion (Figs. 3 and 4). Lymphocytes may or may not be present in the tumor mass. When present, they are diffusely scattered without forming lymphoid follicles as in the case of Warthin's tumor.

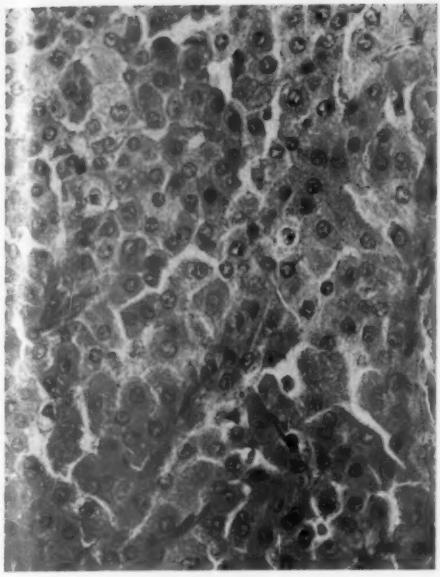

Fig. 1.

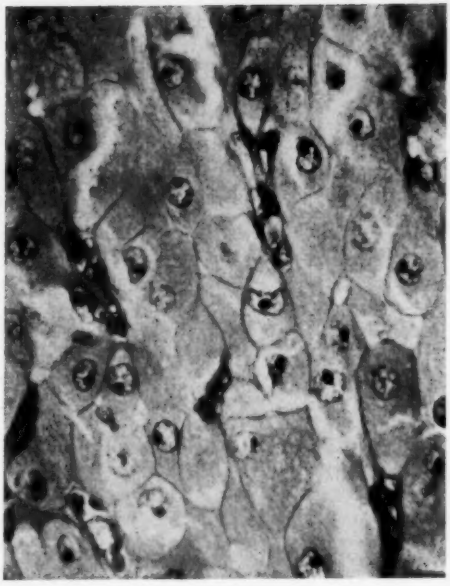

Fig. 2.

Fig. 1.—Photomicrograph of oncocytoma of parotid gland showing cordlike arrangement of cells.

Fig. 2.—Photomicrograph (higher magnification) showing oxyphilic granular cells with distinct cell boundaries and vesicular nuclei.

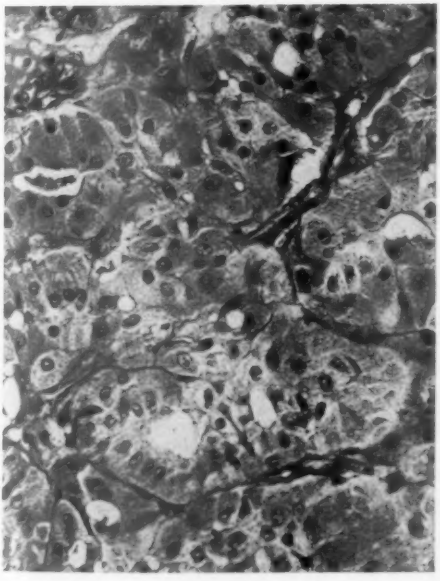

Fig. 3.

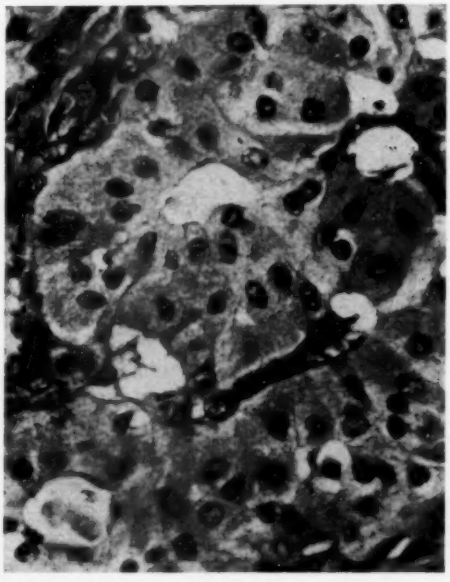

Fig. 4.

Fig. 3.—Photomicrograph of parotid adenoma. Note oxyphilic granular cells forming tubular and acinar structures.

Fig. 4.—Photomicrograph showing tubular and acinar structures at a higher magnification.

TABLE I

NAME OF AUTHORS	YEAR PUBLISHED	AGE	SEX	RACE	SITE	SIDE	SIZE (CM.)	DURATION
1. Chaudhry and Gorlin	1957	66	F	W	Parotid	L	3 × 2 × 2	4 years
2. Chaudhry and Gorlin	1957	62	M	W	Parotid	L	5 × 4 × 1.5	20 years
3. Chaudhry and Gorlin	1957	50	F	W	Parotid	R	—	2 years
4. Chaudhry and Gorlin	1957	74	F	W	Parotid	R	3 × 2.4 × 2	10 years
5. Schafer et al.[18]	1956	53	F	W	Parotid	L	3 × 2.5 × 1.5	—
6. Feroldi et al.[21]	1955	81	F	W	Parotid	–		20 years
7. Berkheiser et al.[12]	1954	53	F	W	Parotid	L	4.5 × 4.5 × 4.5	6 months
8. Boley et al.[22]	1954	65	F	W	Parotid	R and L	Diffused	—
9. Christopherson[23]	1949	67	M	Negro	Parotid	R	7 × 5 × 3.5	2 years
10. Meza-Chavez[9]	1949	52	M	W	Parotid	L	3.5 × 1.0 × 1.5	2½ years
11. Meza-Chavez[9]	1949	72	F	W	Parotid	R	8 × 5 × 2.5	7-10 years
12. Meza-Chavez[9]	1949	–	–	–	—	–	2 × 2 × 2	—
13. Meza-Chavez[9]	1949	60	F	W	Parotid	L	4.5 × 4.5	21 years
14. Meza-Chavez[9]	1949	32	M	W	Parotid	–	1.5 × 1.5 × 1.5	—
15. Stump[8]	1949	53	F	–	Parotid	L	2.5 × 2.2 × 1.8	1½ years
16. Lloyd[24]	1946	59	F	–	Parotid	–	3 × 3 × 3	—
17. Blair and Olch[25]	1944	–	–	–	—	–	—	—
18. Ackerman[17]	1943	76	M	W	Parotid	L	2 × 2 × 2	Several months
19. McFarland[26]	1942	74	F	W	Parotid	L	—	—
20. Skorpil[6]	1940	83	F	–	Parotid	–	—	—
21. Gruenfeld et al.[15]	1936	68	F	W	Parotid	R	3 × 4 × 2	3 years
22. Harris[16]	1936	70	M	W	Parotid	R	3 × 4 × 4	1 year
23. Ahlbom[27]	1935	59	M	–	Palate	L	1½ × 2	4 months
24. Hückel[28]	1930	61	M	–	Parotid	–	—	8 years
25. McFarland[14]	1927	71	M	W	Parotid	–	—	3 years
26. Duplay[13]	1875	47	F	W	Submaxillary	–	—	—

To the above may be added four cases of Bauer and Bauer[10] and three cases of Kirklin[29] which lack clinical data.

Oxyphilic granular cell adenoma is a benign tumor which seldom recurs. There are two cases on record in which recurrence was reported twelve and six years following removal.[14, 20] The recurrences are attributed to either multicentric origin of the neoplasm or its incomplete removal. Since the neoplasm is usually well encapsulated, surgery affords an adequate treatment without any hazard to the facial nerve. Radiation has been tried in only two cases, without much success. There is no conclusive evidence that the tumor may undergo malignant transformation. However, Buxton and associates[20] implied that because of their long duration, three of the nine cases of acidophilic-celled carcinoma in their series could be due to the transformation of benign into malignant tumor.

REVIEW OF REPORTED CASES

The first case of oxyphilic granular cell adenoma was published as far back as 1875 by Duplay,[13] who considered it a variant of mixed salivary gland tumor. McFarland[14] has been credited with the recognition of this tumor as a separate entity, and he designated it as Hürthle-cell adenoma (onchocytoma). His case lacks clinical and histologic description, however. The first well-documented case in this country was reported by Gruenfeld and Jorstad[15] in 1936. Another case was published by Harris[16] in the same year. Ackerman,[17] could collect only seven cases from the literature, and in 1943 he added a new case, bringing the total to eight cases. Three of these cases, however, were rejected as oxyphilic granular cell adenoma by the subsequent investigators. In 1949 Stump[8] reviewed the literature and gathered eleven cases, to which he added another case. He rejected two cases of Stohr and Risak which had been included by Ackerman. Stump, however, included the first case of Hückel[28] which was not mentioned by Ackerman. Meza-Chavez,[9] in 1949, added five cases of oxyphilic granular cell adenoma to the list, but he rejected the case of Steinhardt, which was previously accepted by both Ackerman and Stump. In 1956 Schafer and colleagues[18] from his survey of literature, collected only sixteen authentic cases of this neoplasm and added one case from his own collection.

We have been able to collect twenty-nine authentic cases of oxyphilic granular cell adenoma from the literature and have added four new cases from the files of the Department of Pathology, School of Medicine, and the Division of Surgical Pathology, University Hospital, bringing the total to thirty-three cases. The clinical findings of these cases have been summarized in Table I, and one new case is discussed in detail below. Of the twenty-four cases in which age was specified, nearly 80 per cent occurred in patients between 51 and 80 years of age, as seen in Chart 1. The youngest patient was 32 years old, and the oldest was 83 years old, with an average age of 62.8 years. The neoplasm was found in fifteen women and nine men; thus, it is slightly more common in women, in contrast to papillary cystadenoma lymphomatosum which is five times more common in men. The duration for which the neoplasm had been present before its removal was mentioned in only sixteen cases. It ranged

from four months to twenty-one years, with an average duration of eight years. The location was given in twenty-four cases; twenty-two occurred in the parotid glands, one in the submaxillary gland, and one in the palate, presumably of minor salivary gland origin. Of the seventeen cases in the parotid glands in which the sides were stated, ten occurred on the left, six occurred on the right, and in one case there was bilateral involvement. The largest tumor was found to be 8 by 5 by 2.5 cm. in size. Only one case has been reported in a Negro. There has been no case on record where the tumor has metastasized.

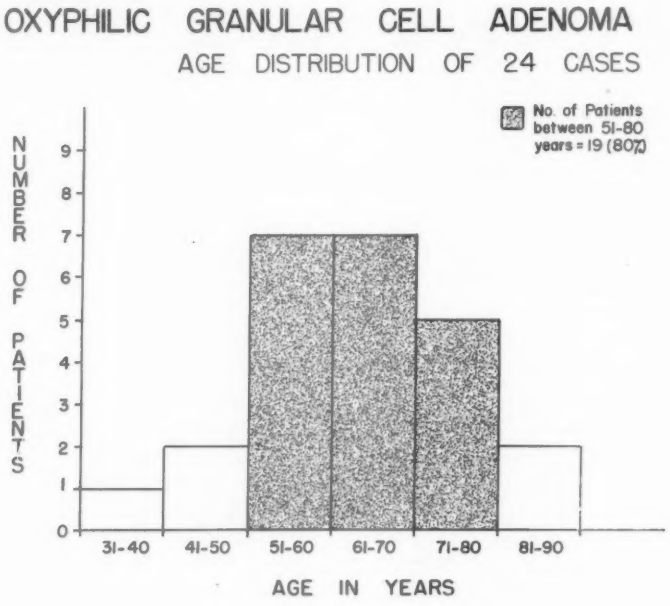

Chart 1.

CASE REPORT

A 74-year-old white woman entered the University Hospital on Nov. 22, 1956, with a firm, nontender, movable mass located anterior to the right ear. The mass had been present for ten years. It had caused her no discomfort and had not grown in size during the past two years. Clinically, the mass was round, discrete, and superficial and was diagnosed as a mixed tumor. In view of the age of the patient, the mobility of the tumor, and its superficial location, it was decided to enucleate it under local anesthesia. A curved incision about 4 cm. in length was made just below the level of the tragus, following the posterior border of the mandible. The tumor mass was easily identifiable under the superficial fascia; it was well encapsulated and shelled out easily by blunt dissection. No nerve fibers could be seen; presumably the nerve was lying deep to the tumor mass. Grossly, the tumor was well encapsulated, firm, nodular, and measured 3 by 2.4 by 2 cm. The cut surface was grayish white in color and was divided into several lobules. Microscopically, the neoplasm was surrounded by an incomplete connective tissue capsule, outside of which there was a small amount of parotid gland as well as clusters of lymphocytes

Fig. 5.—Photomicrograph showing characteristic lobulated pattern of oxyphilic granular cell adenoma of the parotid gland.

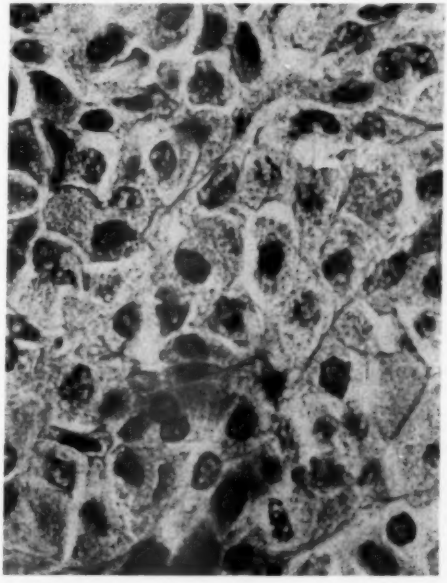

Fig. 6.—Photomicrograph of oxyphilic granular cell adenoma of the parotid gland with large cells, granular cytoplasm, and vesicular nuclei.

without any germinal centers. The lobules were separated by connective tissue septa (Fig. 5). Each lobule was made up of sheets of large epithelial cells of different sizes and shapes and contained abundant eosinophilic granular cytoplasm. The cells had distinct cell boundaries except at places where they were tightly packed (Fig. 6). The nuclei were usually oval and vesicular and had a prominent nucleolus. Throughout the section there were some cells which stained more intensely. They were elongated, round, or irregular cells with hyperchromatic, indented nuclei in which no nucleolus could be seen. The interlobular connective tissue was hyalinized and infiltrated with epithelial cells, similar to mixed salivary gland tumor. However, no mucoid or pseudocartilaginous components of mixed salivary gland tumor were present.

DISCUSSION

The neoplasm has been called by several names in the literature. The term "oncocytoma" was first introduced by Jaffe[19] in 1932 with reference to a neoplasm most commonly known as papillary cystadenoma lymphomatosum (Warthin's tumor) or adenolymphoma. Since the term "oncocytoma" did not explain fully the histologic components of Warthin's tumor, the use of this name for the latter tumor has been abandoned. Ackerman suggested that the term "oncocytoma" should be reserved for tumors made up of large acidophilic granular cells, the so-called "oncocytes" of Hamperl. Meza-Chavez objected to the term "oncocytoma," which is based on the word "oncocyte." According to him, the term is confusing because the Greek root *onkos* has been used extensively for other medical terms with the connotation "pertaining to tumor." Harris introduced the name "pyknocytoma" for this neoplasm in order to differentiate it from Warthin's tumor, which Jaffe had called oncocytoma, and also because of the apparent pyknotic appearance of the nuclei in some cells as suggested by Zimmermann. Since this name was based on an erroneous impression, it has not been well accepted. Meza-Chavez proposed the name oxyphilic granular cell adenoma. Buxton and associates[20] preferred to call it acidophilic granular cell adenoma. Both terms are descriptive of the histologic features of this neoplasm and, therefore, are quite appropriate.

Thankfully acknowledged to Dean W. H. Crawford for his generous help and encouragement in this investigation.

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ENDODONTICS

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ONE-STEP REPRODUCTION OF RADIOGRAPHS

John Philpot, Toronto, Ontario

THERE are many occasions when endodontists and other specialists require a slide of a periapical or an extraoral radiograph which, in itself, is either too dense or of an irregular size for projection by standard apparatus. This incurs added expense and the inconvenience of having copies made commercially. This article is intended, therefore, to enlighten the practitioner on how this may be undertaken by himself with the aid of the circle-flash.

Many dental practitioners appreciate and probably use the circle-flash in conjunction with a 35 mm. camera but do not realize that this apparatus, with very little outlay, can be adapted for producing high-quality reproductions of intraoral and even larger originals when required.

The sensitive material used for this work is Daylight Kodachrome or an equivalent film, and the apparatus is constructed from the following:

1. A sheet of opal glass, 2 inches greater than the largest film to be copied. (Check for complete opalescence; the type used in an x-ray viewbox is usually satisfactory.)
2. One porcelain or plastic lampholder and one 100 watt lamp.
3. One feed-through switch.
4. A 5 foot length of light cord (5 amperes).
5. A small supply of lumber and hardware.
6. A series of x-ray mounts set in opaque masks, the size of which should match the opal glass (Fig. 2).

The apparatus should be assembled as shown in Fig. 1. No detail of construction has been given, as this apparatus will be used for different-sized radiographs and to suit varying space limitations. However, Fig. 1 shows the basic design which can be adapted to suit specific requirements.

From the Department of Visual Aids, Faculty of Dentistry, University of Toronto.

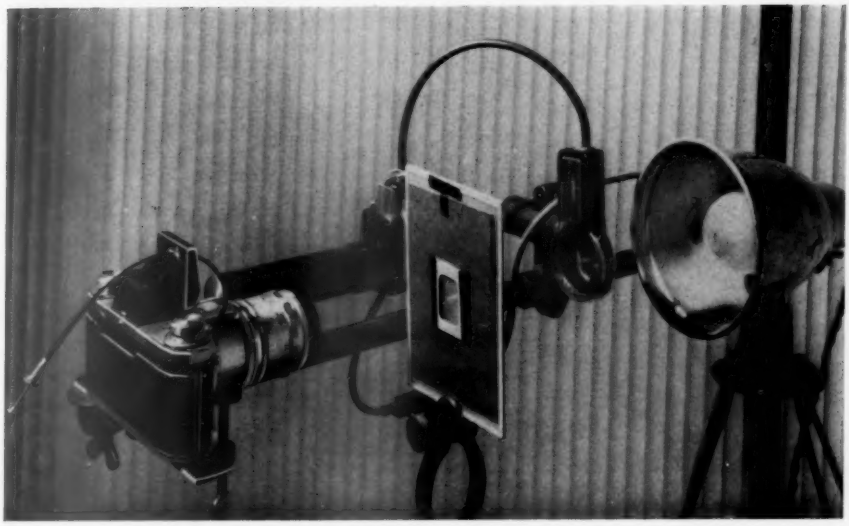

Fig. 1.—Apparatus assembled.

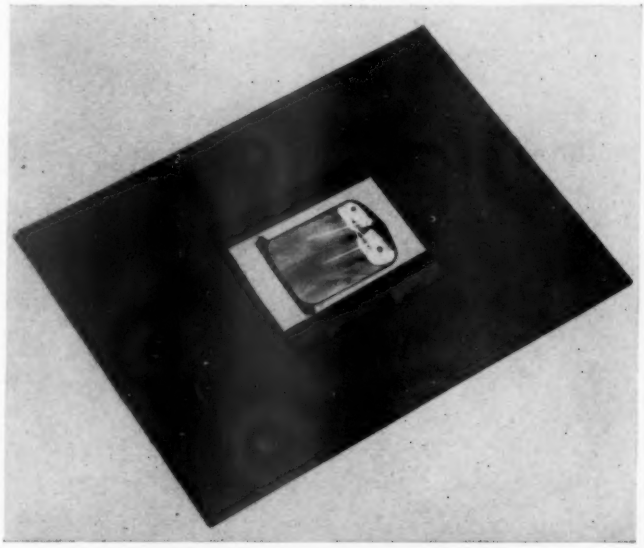

Fig. 2.—Mask showing x-ray film in mount.

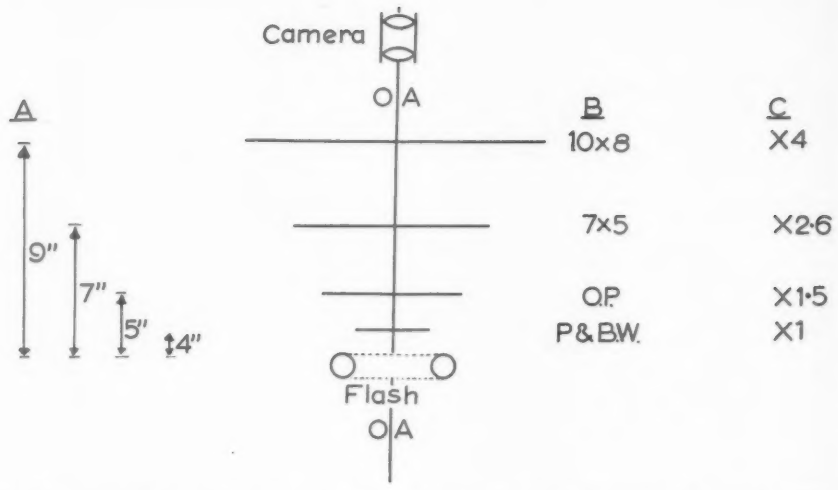

Fig. 3.—*A*, Flash-to-film distances; *B*, x-ray film sizes; *C*, exposure factors; *OA*, optical axis; *OP*, occlusal plane films; *P & BW*, periapical and bite wing films.

Fig. 4.—Comparison guide.

When the apparatus has been assembled, a series of test exposures are made in the following manner:

1. The mask holding the radiograph to be copied is placed on the opal glass.
2. The 100 watt lamp is turned on and the room lights are dimmed (one 40 watt lamp at not less than ten feet from apparatus).
3. The camera is adjusted, with the use of extension rings, to fill the picture area at a sharp focus.
4. Place the Circle-flash behind or beneath the opal glass at the correct distance (Fig. 3) on the same axis as the camera lens and radiograph.
5. Turn off the 100 watt lamp.
6. Make a series of progressive exposures—for example, f2, f2.8, f4, f5.6, f8, f11, at one/twenty-fifth of a second, keeping a record of each exposure made. If the film is larger than 5 by 7 inches it is advisable to set the camera shutter for a time exposure and fire the flash twice manually by means of the test switch provided.
7. When the film has been processed, ascertain which is the correct exposure and retain the data as a guide for all future exposures.

Note: If the distance between the Circle-flash and the radiograph is changed, it will affect the f number setting. (See Fig. 3, exposure factor.)

Generally speaking, radiographs fall into three or four categories varying from light to very dark. A useful comparison guide can be made by mounting some x-rays of varying density for which the correct f number has been ascertained and recorded (Fig. 4).

The data given in this article regarding exposure and distances are intended as an approximate guide for this technique, which will produce consistent and remarkably accurate reproductions that are difficult to achieve by the conventional "negative-positive" technique.

BACTERIOLOGY

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DESCRIPTIONS OF ACTINOMYCETES AND OTHER PLEOMORPHIC ORGANISMS RECOVERED FROM PIGMENTED CARIOUS LESIONS OF THE DENTINE OF HUMAN TEETH

Masao Onisi, D.M.Sc., and James Nuckolls,† San Francisco, Calif.*

WE[26] REPORTED in 1954 that eighty-six pleomorphic strains isolated from pigmented carious lesions of the dentine of human teeth could be divided into five types according to the colonial characters, cellular morphology, and Gram staining at the initial isolation. In order to establish the flora of carious lesions of dentine, it is essential to take measures which rule out contamination by microorganisms from tooth surfaces of specimens taken for cultivation. A survey of the literature indicated that antiseptics may be ineffective in establishing surface sterility. Our results indicated that this condition might be achieved by heating teeth in paraffin at 200° C. for suitable periods and that, under these conditions, organisms in the interior of the tooth were not invariably killed. Since in this investigation we were primarily concerned with a study of bacteria which are found at the innermost part of the preclinical and clinical lesions, destruction of the organisms of the outer areas of the lesions did not seem to be important, as they were considered to be secondary contaminants. The method was important, and it is outlined briefly as follows: Specimen teeth were split through carious lesions with sterile nippers. Samples were then ground from the split tooth surfaces with sterile burs. Samples so collected were used to inoculate plates of suitable media. All of these operations were carried out under aseptic conditions. In material from representative preclinical and early clinical caries which had been subjected to the foregoing sterilization and collection procedures, highly pleomorphic bacteria predominated. Lactobacilli, streptococci, micrococci, and other forms were less frequently observed. The form most frequently isolated appeared to have morphologic similarities to the actinomycetes. A description of the five

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types of bacteria is presented in this article. The bacterial types discussed herein are of interest because of the attention devoted to prevention of contamination in their isolation and because they were found in material from relatively early dentine caries.

METHODS

The following characteristics were made a basis for description of the five types of bacteria:

Morphology was observed when the bacteria were grown in nutrient broth or agar in ordinary plate or tube cultures or on a warm stage incubator. It was revealed by direct microscopic examination or by the use of various stains. Cell walls, cytoplasm, chromatinic bodies, endospores, and flagella were stained as follows: Dyar's[10] method for cell-wall staining; Robinow's[29] acid-Giemsa method for staining the chromatinic body after a preliminary comparison with the ribonuclease method; the Schaeffer-Fulton[32] modification of Wirtz's method for endospores; Leifson's[19] flagella stain; and phenolic erythrosine for protoplasm.

In the warm-stage observations, postfission movements were avoided as much as possible by the use of a small culture cell of dental sheet wax mounted on a slide and covered with a cover glass which had a layer of medium of a thickness that just permitted space for the growth of the bacteria. Observations at high, dry magnification were recorded by sketches or photography. For permanent preparations, growth of bacteria under warm-stage observation was stopped at any desired condition and impression smears were made. Another impression preparation was made directly from a small block from agar plate for comparison. Formalin (6.5 per cent) in ethanol or 95 per cent ethanol was used in fixation. In preliminary examination, the more concentrated the formaldehyde, the easier the agar block could be peeled away from the slide surface, but formaldehyde obscured chromatinic structure when stained by Giemsa's solution or other basic dyes.

Other morphologic observations were performed with young cultures grown on brain-heart infusion at 37° C. Although the eighty-six pleomorphic strains received most attention, better known strains (*Lactobacillus acidophilus* Hill #12, *L. acidophilus* Harrison A. T. C. 4646, *Nocardia asteroides* Conand, *N. asteroides* Kessel, *Corynebacterium pseudodiphthericum, Enterococcus, Escherichia coli, Proteus* X19, *Staphylococcus aureus, Sarcina lutea*) were used as controls.

Formation of certain *products* or *enzymes* was examined in some detail. Liquefaction of gelatin was established by testing whether or not gelatin was solid when refrigerated after several days' incubation at 37° C. Action on nitrates was tested by nitrite reagents and zinc dust. Final pH of 1 per cent glucose broth cultures was measured by means of the glass electrode (Coleman). Formation of gas in carbohydrate media was tested with Durham tubes. Acid was indicated by means of bromeresol purple. Utilization of sodium lactate was determined in order to test the identity of the pleomorphic strains. Cell suspensions were prepared as follows: Ten milliliters of 0.5

per cent (w/v) lactate in a brain-heart infusion received one loop of a suitable strain. Incubation for two days at 37° C. followed. The 10 ml. culture thus prepared was added to 90 ml. of the same media and again incubated for two days at 37° C. After the cells were harvested by centrifugation, they were washed once with a 0.5 per cent (w/v) sodium lactate phosphate buffer and incubated for five days at 37° C. under a layer of petrolatum. Cleavage of lactate was determined from differences of lactate concentrations before and after fermentation. The method of lactate determination reported by Barker and Summerson[3] was used. Carbohydrates tested were glucose, lactose, sucrose, maltose, and raffinose. The only glucoside tested was salicin. The sugar alcohols used as substrates for fermentation were mannitol and dulcitol. Milk coagulation and peptonization were studied. Tests were made for the presence of catalase and the formation of hydrogen sulfide and indole.

DESCRIPTION

Although we[26] had classified our eighty-six pleomorphic strains in five types—I, II, III, IV, and V—on the basis of a preliminary study, it was necessary as a result of this present work to subdivide some of these groups even further. The basis for this subdivision was the action of the bacteria on lactose and the final pH of cultures in glucose broth (Table I).

In order that this classification might be consistent with that presented in our earlier article,[26] our type numbers do not correspond precisely with the action of the bacteria on lactose. Type numbers are assigned in accordance with the terminology of our previous article. There were two types that were able to utilize lactose. One of these types consisted of ten strains which manifested the final pH value in glucose broth of 4.0. This type was known as Type I. The second type that utilized lactose produced a final pH value of 4.5 when incubated in glucose broth. This type consisted of nineteen strains and was known as Type IIIA. There were four types that did not utilize lactose.

The first of these types, consisting of seventeen strains, did not alter the final pH in glucose broth. This was known as Type II. The final pH value observed in cultures of Type II was approximately 7.0. The second type, Type IIIB consisting of twenty-two strains, produced a final pH in glucose broth of 4.8. Type IIIC, six strains, reduced the final pH in glucose broth to from 5.5 to 6.5. Type IV, consisting also of six strains, apparently did not utilize glucose but incubation in glucose broth resulted in a final pH value of 8.0. Of these various types, IIIC and V were variable, but each of the other groups of strains showed fairly constant characteristics. Type V, which is not mentioned above, consisted of six strains that were morphologically and biochemically distinct from each other. Group V, therefore, was a miscellaneous group. Because of the variability of Types IIIC and V, they have not been included in our present list of descriptions. In the descriptions which follow, we have made no attempt to assign species names, although in our discussion later on we consider a tentative classification of these types of bacteria.

Type I.—

Morphology: Smears from agar cultures show gram-positive rods, but in broth cultures the cells appear as gram-positive streptococci. Flagella, capsules, or endospores could not be demonstrated by our method.

TABLE I. UTILIZATION OF LACTATE AND FINAL pH IN 1 PER CENT GLUCOSE BROTH

STRAINS	REMAINING LACTATE AFTER FERMENTATION (PER CENT)	FINAL pH IN 1% GLUCOSE BROTH
L. acidophilus Hill 1	97	4.0
L. acidophilus Hill 12	95	4.0
L. acidophilus Hill 20	95	4.0
L. acidophilus Hadley 4646	94	3.9
L. acidophilus Harrison 3A1S	96	3.9
Escherichia coli	57	4.7
Type I		
8	94	4.0
10	100	4.1
24	105	4.0
71	97	4.2
102	98	4.0
104	93	4.0
107	95	4.0
119	95	4.0
122	91	4.0
Type IIIA		
3	79	4.4
4	57	4.4
17	86	4.4
23	86	4.5
101	61	4.3
120	92	5.0
124	86	4.4
Type IIIB		
1	36	4.8
2	49	4.8
7	79	4.8
12	64	5.0
14	69	5.0
21	79	5.0
22	86	5.0
112	50	4.9
128	83	4.9

Initial observation of cells freshly transferred from broth to the solid medium used for warm-stage observation gave the impression of long chains resembling beaded strings. Shortly after the onset of growth, these chains appeared to branch. These branching chains curved back gradually until they touched the chain initially present, usually at a point near the start of the branch. Continuation of the process of branching and curving leads to a meshwork of interlocking chain which becomes so close-knit after a time that reproduction may no longer be followed (Fig. 1). The net result of this process is a granule. From this granule, long threads grow out more slowly into the medium and only in a horizontal direction. These two methods of reproduction may explain the colonial appearance noted below.

Fig. 2.

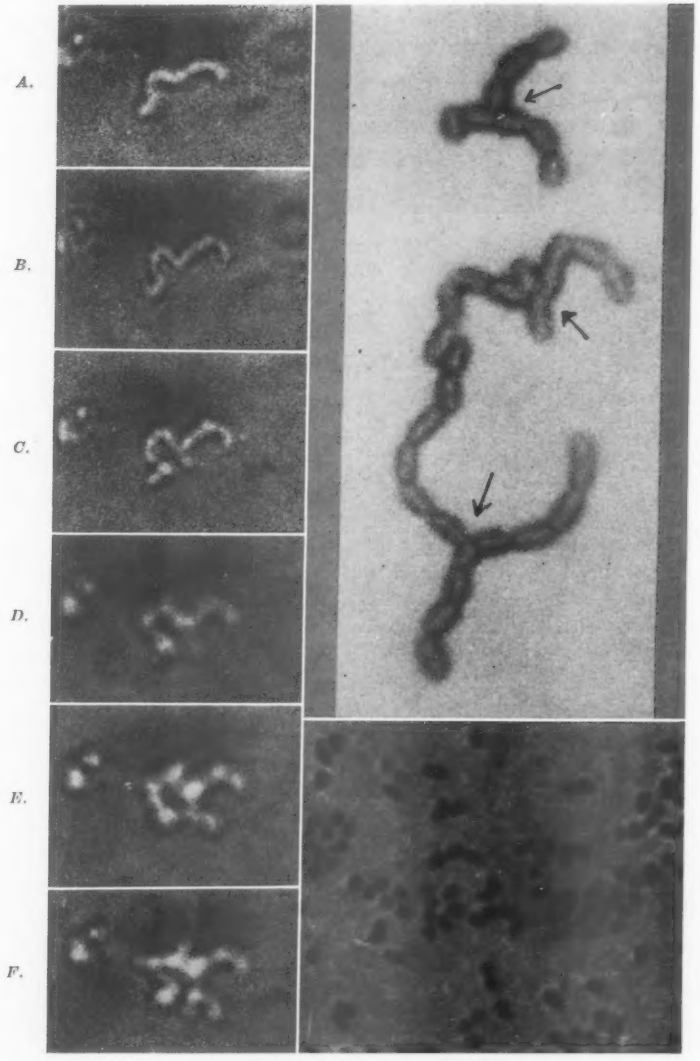

Fig. 1. Fig. 3.

Fig. 1.—Warm-stage observation of initial growth of a Type I strain, 711; photographed by Tiyoda objective ×40 and ×15 and enlarged at photographing and printing. *A* to *F* are taken at 0, 1, 2, 3, 5, and 6 hours, respectively, under 37° C. warm condition. The rate of enlargement beyond the optical system of the microscope is the same in the figures that follow.

Fig. 2.—Type I strain, 241, stained by Dyar's method for cell walls. Tiyoda objective ×90 oil and ocular ×15.

Fig. 3.—Type I strain, 241, stained by Robinow's method for chromatinic bodies. Tiyoda objective ×90 oil and ocular ×15.

Impression smears made of very young cultures reveal the shape of the individual cell under warm-stage cultivation as a more or less tapered cylinder. Some of these cells have short branches which appear to be separated by a septum (Fig. 2). Some cells are present in a ramifying chain as streptococci. A round chromatinic body is located at the center of young cells (Fig. 3).

Colonial morphology: Colonies on agar are circular and umbonate with a rough central area and a translucent outer area which is smooth, flat, and often concentrically ringed with an entire margin. These colonies may change at times to a smooth, raised form or to a rough, convex type with an extremely narrow and flat margin. The central rough area is adherent to the agar and is not easy to emulsify, while the marginal smooth part is more butyrous in consistency. Development of colonies requires three or more days at 37° C. in primary cultures, but after subculture on usual media colonial development is much more rapid.

Agar stroke: Growth on agar stroke is rather scanty, diffuse, and glistening. Stroke is colorless. Odor is absent.

Nutrient broth: In nutrient broth, a moderate sediment is formed; turbidity is moderate to slight. There is no surface growth.

Formation of certain products or enzymes: Catalase, indole, and hydrogen sulfide could not be detected. Milk was curdled within one or two days without retraction of clot. Gelatin was not liquefied. Nitrite was not produced from nitrate under the conditions of the experiment. Acid, but no gas, was produced from glucose, lactose, sucrose, maltose, mannitol, salicin, raffinose, and inulin. There was no indication of utilization of dulcitol. Lactate was not fermented (Table I). Final pH in glucose broth of all the strains reached 4.0 almost within twenty-four hours (Tables I and IV).

Type II.—

Morphology: Vegetative cells are gram-positive short diphtheroids or pleomorphic cocci, often in irregular or palisade arrangement, without spores, flagella, or capsules. The diphtheroid cells first elongate and then divide into two parts which are not always of equal size. New cells manifested extremely active postfission movement, leading to an irregular cellular arrangement of the initial colony.

The results of cell-wall or cytoplasmic staining showed that the small pleomorphic cocci contain two cellular bodies and the diphtheroid cells contained from five to six or more of these bodies (Figs. 4 and 5). The chromatinic body of young cells was round and located centrally in the cell.

Colonial morphology: The colonies of this type are punctiform, raised with the smooth surface and entire margin as grown on agar. Growth to maximal size requires about twenty-four hours.

Agar stroke: Growth of the agar stroke is very scanty and diffuse. These cultures lack odor, and the growth has a butyrous consistency.

Nutrient broth: Growth in broth yields light turbidity and scanty sediment. There is no visible pellicle. These broth cultures are odorless.

Formation of certain products or enzymes: Catalase was formed, but indole and hydrogen sulfide were not detected. Gelatin was not liquefied. No nitrite was produced from nitrate under the conditions of the experiment. Milk was not coagulated. Neither acid nor gas was formed from certain sugars under the experimental condition (Table IV).

The cultures are aerobic and microaerophilic.

Fig. 4. Fig. 5.

Fig. 4.—Type II strain, 12, stained by Dyar's method for cell walls. Tiyoda objective ×90 oil and ocular ×15.

Fig. 5.—Type II strain, 12, stained by carbol erythrosine method for cytoplasm. Tiyoda objective ×90 oil and ocular ×15.

Type IIIA.—

Morphology: These cells are gram-positive and resemble branched short hyphae without flagella, capsules, or endospores (Fig. 8). Reproduction on the warm stage was very slow. From one end or lateral part of the cell, ordinarily, new growth occurs as a branch (Fig. 6). Staining of cell walls revealed that cells are several times as long as they are broad and that some of the branches are separated from the "mother cells" by septa (Fig. 7). The chromatinic body is spherical and is placed either in the center of the cell or close to the septum (Fig. 9).

Colonial morphology: Agar colonies of this type were circular, pulvinate, entire, smooth, and opaque. These colonies required three days to reach maximum size in primary culture, but upon domestication they grew much faster.

Agar stroke: Growth of the agar stroke was moderate and beaded or filiform. These cultures showed a slight odor which was quite definite. The growth was of butyrous consistency and was colorless.

Nutrient broth: In broth there was a moderate turbidity, abundant compact sediment, but no surface pellicle.

Formation of certain products or enzymes: Indole and hydrogen sulfide were not formed. Eleven of the nineteen strains produced catalase, while ten strains produced nitrite from nitrate. Most, but not all, of the catalase-forming

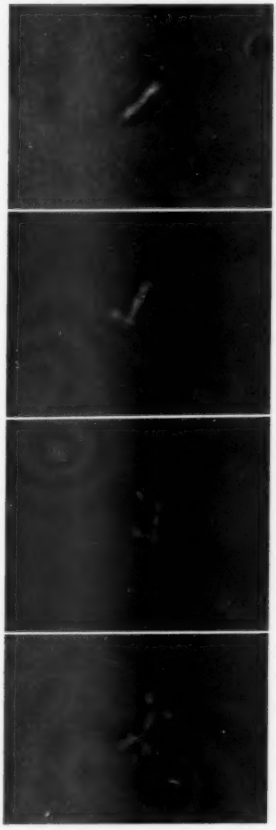

Fig. 6.—Initial growth of a Type IIIA strain, 353, under warm condition. Tiyoda objective ×40 and ocular ×15. *A* to *D* are photographed at 0-, 1-, 2-, and 4-hour incubations, respectively.

strains also reduced nitrate in the same culture. Fourteen strains curdled milk slowly without retraction of the acid clot, and other strains produced acid but no clot. Gelatin was liquefied by some, but not all, of the strains. Acid, but no gas, was formed from glucose, lactose, sucrose, maltose, salicin, raffinose, and inulin. Neither acid nor gas was produced from mannitol and dulcitol (Tables II and IV). Final pH of 1 per cent glucose broth averaged 4.5 within three to four days. Lactate was fermented (Table IV).

These cultures are all microaerophilic facultative anaerobes.

TABLE II. BACTERIOLOGIC CHARACTERISTICS OF TYPE IIIA

STRAINS	35	44	46	49	56	65	74	27	31	37	68	101	105	120	124	132	17	23	3
Catalase production	+	+	+	+	±	−	−	+	+	+	+	−	−	−	+	+	−	−	−
H_2S production	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Indole production	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Nitrite from nitrate	+	+	+	+	+	−	−	+	+	+	+	−	−	−	−	−	−	−	−
Acid coagulation of milk	+	±	−	−	−	+	+	+	+	+	+	+	−	+	+	−	+	+	+
Gelatin liquefaction	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Final pH in 1% glucose broth	4.5	4.4	4.5	4.4	4.5	4.5	4.3	4.6	4.5	4.5	4.7	4.5	4.6	5.0	4.4	4.5	4.4	4.5	4.5
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±	+
Salicin	+	+	+	+	+	+	+	+	+	±	±	+	+	+	+	+	−	−	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inulin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dulcite	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

TABLE III. BACTERIOLOGIC CHARACTERISTICS OF TYPE IIIB

STRAINS	19	25	112	12	14	21	32	53	60	64	72	111	128	1	7	13	36	76	108	118	28	42
Catalase production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S production	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Indole production	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Nitrite from nitrate	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+
Acid coagulation of milk	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
Gelatin liquefaction	+	+	+	+	+	+	+	+	−	−	+	+	−	+	−	+	−	+	−	+	+	+
Final pH in 1% glucose broth	4.7	4.7	4.9	5.0	5.0	5.0	4.8	5.0	4.7	4.8	4.5	4.7	4.6	4.8	4.8	4.8	4.7	4.7	4.8	4.9	5.3	4.7
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	±	+	+	+	+	+	±	+	±	+	+	+	+	+
Mannitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−
Salicin	±	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Raffinose	±	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Inulin	±	+	±	±	±	±	±	±	±	+	+	+	+	±	±	±	±	±	±	±	−	−
Dulcite	±	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Fig. 7.

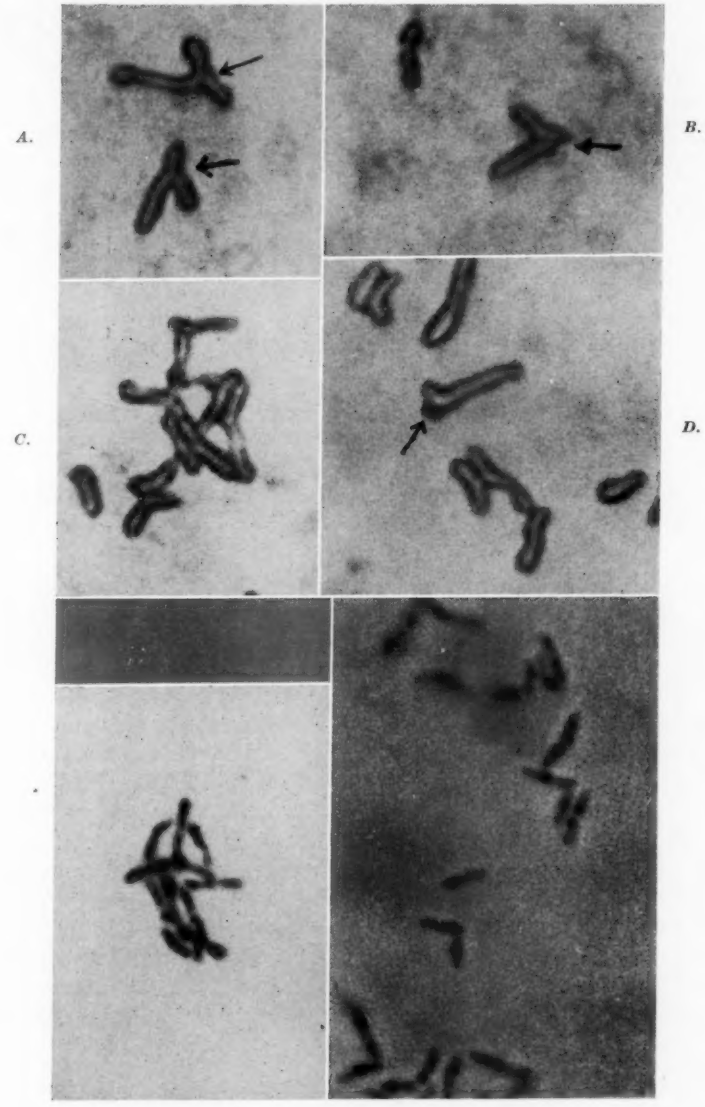

Fig. 8. Fig. 9.

Fig. 7.—Type IIIA strain, 353, stained by Dyar's method for cell walls. Tiyoda objective ×90 oil and ocular ×15.

Fig. 8.—Type IIIA strain, 353, stained by carbol erythrosine method for cytoplasm. Tiyoda objective ×90 oil and ocular ×15.

Fig. 9.—Type IIIA strain, 353, stained by Robinow's method for chromatinic bodies. Tiyoda objective ×90 oil and ocular ×15.

TABLE IV

CHARACTERISTIC	TYPE I (10 STRAINS)	TYPE II (17 STRAINS)	TYPE IV (6 STRAINS)	TYPE IIIA (19 STRAINS)	TYPE IIIB (22 STRAINS)
Catalase production	−	+	+	±	+
H_2S production	−	−	−	−	−
Indole production	−	−	−	−	−
Nitrite from nitrate	−	−	−	±	+
Acid coagulation of milk	+	−	−	±	−
Rennet and peptonization		−	+	−	−
Gelatin liquefaction	−	−	+	−	±
Final pH in 1% glucose broth	4.0	−	5.3 → 8.1	4.5	4.8
Glucose	+	−	+	+	+
Lactose	+	−	−	+	−
Sucrose	+	−	−	+	+
Maltose	+	−	−	+	+
Mannitol	+	−	+	−	−
Salicin	+	−	+	+	−
Raffinose	+	−	−	+	−
Inulin	+	−	−	+	±
Dulcite	−	−	−	−	−

Type IIIB.—

Morphology: These cells are all gram-positive branched diphtheroids or filamentous forms. Flagella, capsules, endospores, or conidiospores were not observed.

During several months of laboratory culture, the gross morphology of some of these cultures has changed; short mycelial types have been clearly distinguished from the original longer mycelial types. Warm-stage observation of the longer types of filaments showed that many branches grew out of the main filament (Fig. 10). Cell-wall and cytoplasmic staining showed that these filamentous bodies were composed of many cells whose breadth was approximately the same as their length at the beginning of their development. Lateral branches were also separated by septa from the cell of origin (Figs. 11 and 14). The center of a colony in the early stage of growth or the peripheral parts of older colonies appeared as a mass of polygonal short cells (Fig. 11). Chromatinic bodies of young cells were difficult to stain, while in older cells they were spherical and central. Reproduction of the short filamentous type was cubical by repeated lateral expansion and fission. Initial microcolonies were indistinguishable from staphylococcus colonies. Chromatinic (Fig. 13), cell-wall (Fig. 12), and cytoplasmic staining (Fig. 15) of colonies of the short filamentous type indicated that they resembled segmented or aged colonies of the long filamentous type.

Colonial morphology: Colonies on agar were soft or tough, but at times there was evidence of a T-S dissociation. Colonies of the tough type were irregular and opaque, while the soft ones were entire, pulvinate, and opaque.

Agar stroke: Transplants from soft colonies were more abundant than those from Type IIIA and were either beaded or filiform. The consistency was butyrous and slightly adherent to the agar. Strokes from the tough type were

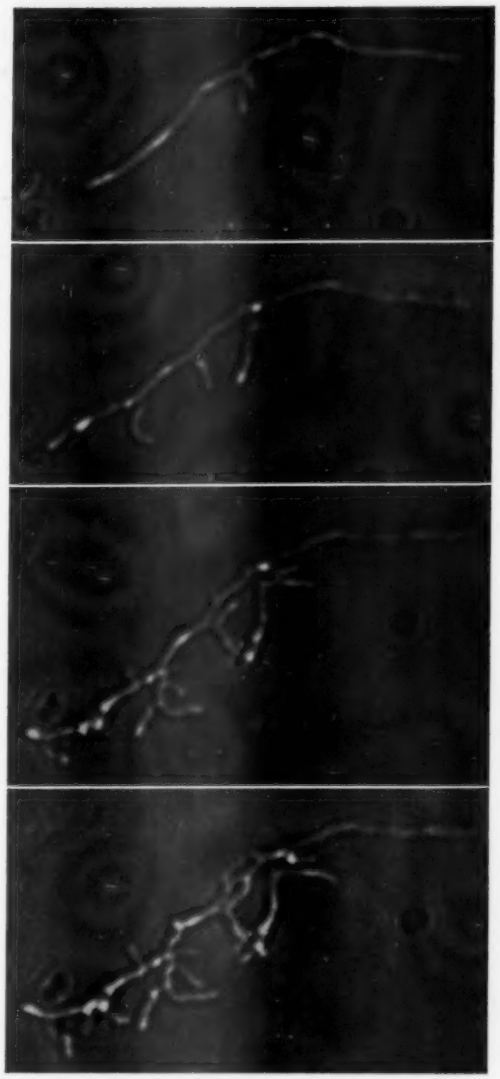

Fig. 10.—Initial growth of Type IIIB strain, 143, by warm stage observation. Tiyoda objective ×40 and ocular ×15. *A* to *D* are taken at 0-, 3-, 6-, and 7-hour incubations, respectively.

abundant and beaded. This type of growth was of a hard consistency and adhered to the agar. Neither type produced colored growth, but there was a decided odor.

Nutrient broth: Inoculations from smooth types of colonies produce moderate turbidity, abundant compact sediment, and a decided odor. The rough colonies give rise to almost no turbidity, a granular sediment, and a moderate odor. In no instance was a pellicle seen.

Fig. 11.

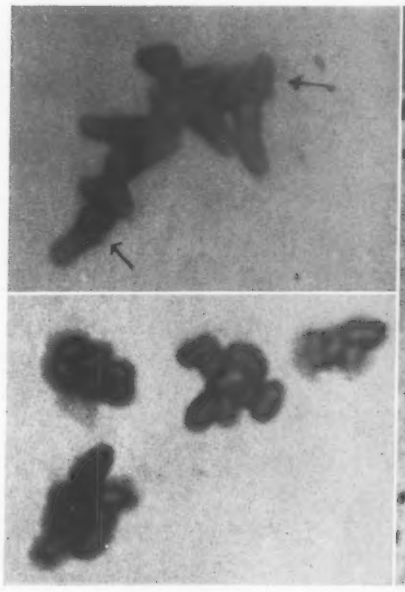

Fig. 12.

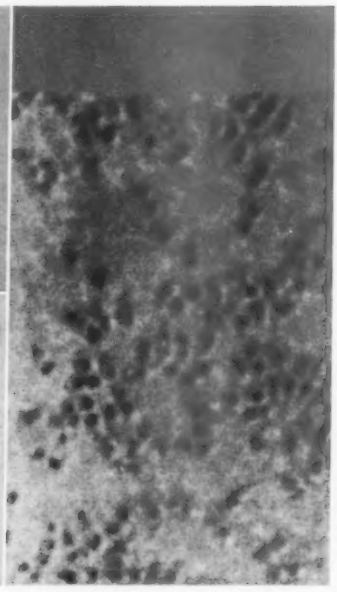

Fig. 13.

Fig. 11.—Type IIIB strain, 134, stained by Dyar's method for cell walls. Tiyoda objective ×90 oil and ocular ×15.

Fig. 12.—Type IIIB strain, 74, short filamentous strain, stained by Dyar's method for cell walls. Tiyoda objective ×90 oil and ocular ×15.

Fig. 13.—Type IIIB strain, 74, stained by Robinow's method for chromatinic bodies. Tiyoda objective ×90 oil and ocular ×15.

Formation of certain products and enzymes: Cultures of this type did not form indole or hydrogen sulfide and did not curdle milk. The catalase test was positive. Twenty strains (91 per cent) produced nitrite from nitrate; sixteen strains (73 per cent) liquefied gelatin in ten to fifteen days at 38° C. Acid, but no gas, was produced from glucose, sucrose, maltose, and inulin. As a rule, neither acid nor gas was produced from lactose, mannitol, salicin, raffinose, and dulcitol. Three strains seem to have a slight action on salicin, raffinose, and dulcitol (Table III). The pH in glucose broth after three to four days averaged 4.8 and lactate was fermented (Tables I and III). The strains of this type were all microaerophilic or facultative anaerobic.

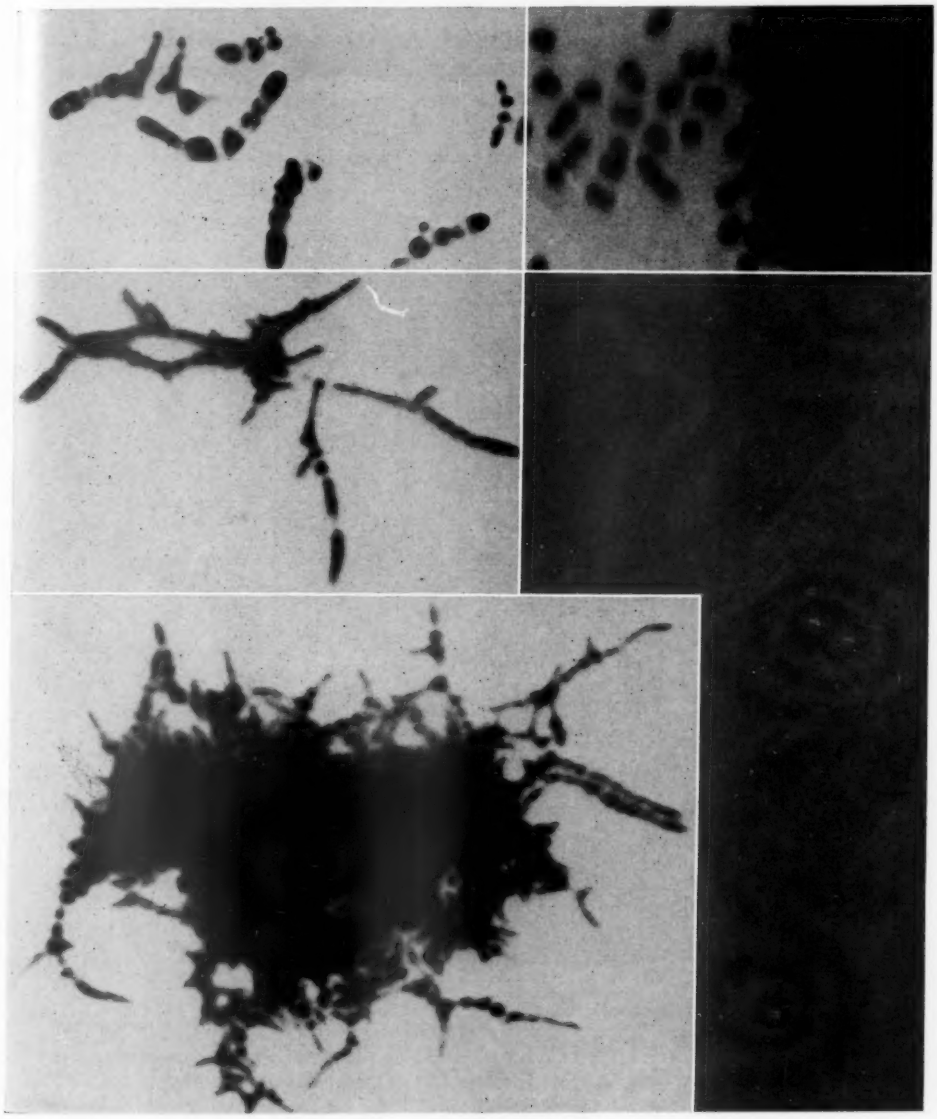

Fig. 14.—Type IIIB strain, 353, stained by carbol erythrosine method for cytoplasm. Impression smears are taken from different places on an agar plate at different incubation times: *A* at 2 hours, *B* at 5 hours, *C* at 8 hours, and *D* at 24 hours. Tiyoda objective ×90 oil and ocular ×15.

Type IV.—

Morphology: Longer rods combined with filaments were seen. The gram stain was variable. Cells seemed to be flexible, and staining showed peritrichous flagella. Capsules were absent, but central or eccentric ellipsoidal endospores were present in the shorter rods.

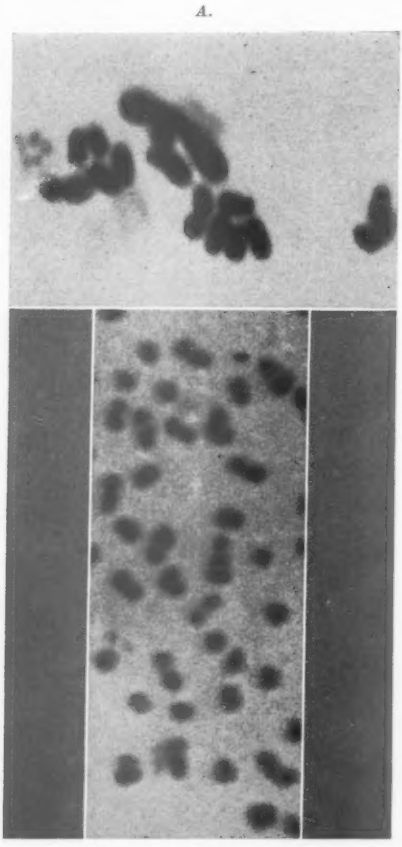

Fig. 15.—Type IIIB strain, 74, stained by carbol erythrosine method for cytoplasm. Impression smears *A* and *B* were taken from different places on an agar plate at 2- and 24-hour incubation times. Tiyoda objective ×90 oil and ocular ×15.

In warm-stage observations the bacteria were observed to elongate initially, but after a short time they suddenly broke up into many rods about 8 μ in length. These rods were motile and swarmed over the agar surface. After a few hours bacterial masses appeared around nonmotile cells. In these masses there are many gram-negative cells, but the still motile bacteria are gram positive. When the agar content of the medium was increased to 2 per cent thread-like growths resembling leptothrix were produced. Spores began to appear in the cultures after five days of incubation.

Impression smears, stained for cell walls, showed the long filaments to be septate. However, the elementary cells were usually long and apparently flexible (Fig. 16). The chromatinic bodies were not round but were ellipsoidal like those of vegetative cells of *Eubacteriales* (Fig. 17).

Colonial morphology: Agar colonies are flat, opalescent, and variable in shape. At times they are smooth with entire or lobate margins. They may also be rough and corded. Colonies were viscid in consistency.

Agar stroke: Growth on slants was viscid, abundant, and spreading. There was no chromogenesis, but a decided odor was present.

Fig. 16.

Fig. 17.

Fig. 16.—Impression smear of Type IV strain, 565, stained with Loeffler's methylene blue solution. Tiyoda objective ×90 oil and ocular ×15.

Fig. 17.—Type IV strain, 565, stained by Robinow's method for chromatinic bodies. Tiyoda objective ×90 oil and ocular ×15.

Nutrient broth: In this medium cultures showed considerable turbidity, a marked pellicle, and an abundant slimy sediment. The odor was strong.

Formation of certain products and enzymes: While, under the experimental conditions, nitrite was not produced from nitrate, the catalase test was strongly positive. The stringy substance was produced in broth, together with an alkaline reaction. Coagulation and subsequent peptonization were frequent in milk. Gelatin was liquefied in three days.

Acid without gas was produced from glucose, mannitol, and salicin. Neither acid nor gas came from lactose, maltose, sucrose, raffinose, inulin, and dulcitol. In glucose broth the pH fell in one or two days to 5.3, but with continued incubation this level increased until a final pH of 8.1 was attained (Table IV). All strains were aerobic.

DISCUSSION

Many pleomorphic strains isolated from natural sources have been looked upon as actinomycetes. Because of a suggestion that the actinomycetes be considered as forming a link between fungi and bacteria, confusion has arisen concerning identification of pleomorphic strains. Evidence of confusion is found in the varied terminology used by different research workers at different times and in the numerous systems of classification, as seen in Waksman's[38] book in which fourteen different systems are listed. There are at least three reasons for this state of affairs: (1) confusion of interrelations of genera in *Actinomycetales,* (2) difficulties of distinguishing between actinomycetes and true bacteria, and (3) variability or mutation of pleomorphic forms.

Under the impact of a series of investigations, there has been gained some idea of how to classify *Actinomycetales.* Ørskov[27] applied the technique of warm-stage observation and defined *Micromonospora.* By means of the same techniques, Jensen[13] defined *Actinomyces.* The concepts of these workers were supported by the cytologic work of Klieneberger-Noble.[15] Umbreit[35] pointed out that the boundaries between *Proactinomyces* and *Corynebacterium* or *Mycobacterium* were indistinct and proposed that *Proactinomyces* be broken up into α and β proactinomyces. Waksman and Henrici[39] divided *Proactinomyces* of Jensen into two genera, *Actinomyces* and *Nocardia,* and proposed the system of classification of *Actinomycetales* which has been adopted in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*[4] More recently, the results of tannic acid violet staining for cell walls may indicate that *Corynebacterium, Mycobacterium,*[5] and *Nocardia* are multicellular coccoid organisms, while *Actinomyces* may be bacillary.[7] Bisset and Moore[8] also separated a new genus, *Jensenia,* from the diphtheroid group and proposed a new system for classification of the actinomycetes, based on their conception of the units of bacterial cytology.

Much confusion still remains, however, concerning the distinction of actinomycetes and other bacteria. Rosenow[31] described strains of streptococci which showed all gradations in morphology from perfectly round cocci to straight and clubbed bacilli. Mellon[21] held an opinion that streptococci are phylogenetically related to the diphtheroids and may have a diphtheroid phase as part of their normal life cycle. These strains were called "diphtheroid streptococci," "diphtheroid phase of streptococci," or "pleomorphic streptococci" and have been reported from endocarditis[1, 18] and cases of urinary diseases.[11, 14] Streptococci isolated from dental materials by Clark[9] and by Tunnicliff and Hammond[34] were also extremely pleomorphic. Appleton,[2] in his textbook, cited that the colonies of *Streptococcus mutans* have the property of adhering closely to the surfaces of teeth, a property stressed by Kliger for

his *Cladothrix placoides* and *Leptothrix*. Krassilnikow[16] claimed that chains formed as a phase of the life cycle of his *Mycobacterium* have some resemblance to streptococci. Coccoid actinomycetes, *Mycococci*,[17] are included in his classification. Schlossmann and Hedman[33] recently found, by means of the electron microscope, that the production of ramifying chains of pleomorphic streptococci was initiated by a mode of reproduction which resembled budding. Difficulties of differentiation between *Lactobacillus bifidus* and actinomycetes were summarized in Rosebury's[30] reviews. In his report of biochemical activities of actinomyces of Group IIB (Ørskov), von Magnus[37] stated that certain acid-producing strains possessed the characteristics of lactic acid bacteria and grouped the former together with the latter under the designation *Lactomyces*. As a matter of fact, confusion appears to be the rule in the identification not only of species but also of genus, family, and even order.

The variations or mutations of these organisms appear to influence not only the species characteristics but also generic or higher characters as well. Novak and Henrici[25] reported a case of transformation from actinomyces to staphylococci and a reverse change. They supported a theory that staphylococci are related to the actinomycetes. Jensen[12] also showed that under the influence of ultraviolet rays, or even spontaneously, two strains of soil actinomyces changed to new types, one of which resembled typical species of streptomyces with a well-developed aerial mycelium and others of which were closer to *Mycobacterium* or *Corynebacterium*. Such variations may lead to indecision as to the order in which to classify a particular organism.

Working as well as one may within the limits of this difficult situation, however, we have been able to make tentative identification of the strains examined in the present work.

Bisset[6] stated that the main difference between the true bacteria and the diphtheroid-coccus group is that the latter return from the mitotic to the resting condition of the nucleus between each cell division, whereas the former retain the mitotic condition throughout the vegetative phase. The shape of the chromatinic body of young bacteria is an important criterion for deciding in which of these two groups to classify an organism. From this criterion, Type IV is clearly *Eubacteriales* and from the character of the flagella and the type of endospore formation as well as other characteristics, it is to be classified in the genus *Bacillus*. Types II, IIIA, and IIIB belong to *Actinomycetales*.[7] Type I is not to be grouped with the *Eubacteriales*. Type I is a rod on solid media but a streptococcus in broth. Cytologic methods and warm-stage observation, however, showed that these "cocci" are threads composed of cylindrical cells. Hence, the streptococcal morphology appears to be an artifact, perhaps caused by routine heat fixation. This type also grows laterally and forms branches (more accurately, budding) or a ramifying chain. This is closer to the behavior of the corynebacteria than to that of the streptococci. Because of this, definite identification with genus is impossible at present. Results of the routine tests showed that this type is similar to *Streptococcus mutans*,[9] which is considered to have only historical interest and is impossible to identify now (Bergey's Manual,[4] p. 336).

Type II had strong postfission movements and was diphtheroid in gross morphology with a central round chromatinic body. It appeared to be multicellular. It probably should be identified with the genus *Corynebacterium.*[7]

Type IIIA could not be distinguished from Type IIIB in gross morphology, but it differed in biochemical and cytologic characteristics. Because of the microaerophilic or facultative anaerobic nature, Type IIIA probably should be identified with *Actinomyces.*[7] From the branching and the coccoid shape of elementary cells of its filaments, Type IIIB may be identified with *Nocardia.*[7] The type which forms short filaments resembles the genus *Corynebacterium,* but results of warm-stage observation indicate that it is closer to the genera *Staphylococcus* or *Sarcina.* This group could not be divided from the long mycelium type by either gross morphology or biochemical reactions at initial isolation. Thus, it may be that the short filamentous type dissociated from the long one. Similar variations in *Nocardia* are often seen in the literature[22, 25] and in the laboratory.

Numerous reports concerning pleomorphic organisms, such as *Lactobacillus acidophilus, L. bifidus,* pleomorphic streptococci, *Cladothrix, Leptothrix,* and actinomycetes, have accumulated in the literature of dental caries bacteriology. Many of these organisms are not identifiable today, but *Lactobacillus acidophilus,* suspected by many to be the most common acid producer in dental caries, has been described as a homofermentative species of *Lactobacillus,*[4] producing only traces of by-products other than lactic acid. Van Dam[36] stated that an inhibitory factor in the lactic acid fermentation is the accumulation of undissociated lactate in a fermentation medium. Even though this has not been accepted, generally as *the* diagnostic factor, it is a fact that lactate is an end product of homofermentative lactic acid bacteria. Pederson and Bagg[28] claimed that the final hydrogen ion concentration among lactic acid bacteria depended on the ratios of the concentrations of the various acids present at the end of fermentation, especially the lactate-acetate ratio. Higher hydrogen ion concentration may often indicate lactate as a major product. Hence, final pH values of cultures, compared under similar experimental conditions, could be significant for identification. According to these conceptions and the results shown in Tables I and IV, Types IIIA and IIIB could be neither homofermentative *Lactobacillus acidophilus* nor *L. bifidus.*

In gross morphology and biochemical reaction, Type IIIB appears identical with actinomycetes isolated by one of us (Onisi[24, 25]) in his early research in dental caries which produced dark brown pigmentation in dentine in in vitro experiment. As pointed out earlier, the largest number of strains described herein fell in Class IIIB. Likewise, in our earlier article[26] it was reported that the organisms most frequently isolated from preclinical caries of the dentine were those of Class III. There may possibly be some relationship between these organisms and caries of the dentine.

SUMMARY

Eighty-six strains isolated from pigmented carious lesions of human teeth were divided into six groups based upon final pH levels in glucose broth and

fermentation of lactose. Within each type, except for two miscellaneous groups, strains showed little variation in results of bacteriologic examinations. Cytologic methods for cell walls and chromatinic bodies in combination with warm-stage observation were very helpful in identifying these eighty-six pleomorphic strains. Of these, twenty-two Type IIIB strains were identified with *Nocardia,* nineteen Type IIIA strains with *Actinomyces,* seventeen Type II strains with *Corynebacterium,* and six Type IV strains with *Bacillus.* There was no category to apply to ten streptococcal-diphtheroid strains of Type I, suspected to be a new genus. Six strains of Type IIIC appeared to be oral actinomycetes (probably *Nocardia*) but were not a homogeneous group. The six remaining strains appeared to be *Eubacteriales* but they were quite variable in morphology and in biochemistry.

We should like to thank Dr. W. E. Hutton for his criticism, Mrs. Barbara K. Stutzman for photographic aid, and Miss Dorothy Comstock for secretarial assistance.

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PROFESSIONAL NEWS ITEMS

American Academy of Dental Medicine

The Academy will hold its thirteenth midannual meeting at the Hotel New Yorker in New York City on Sunday Dec. 7, 1958.

The afternoon scientific session will be devoted to "The Gingival Tissue." Participating in this program will be the following:

Henry M. Goldman, D.M.D., Director of the Riesman Dental Clinic, Beth Israel Hospital, Boston; Professor of Periodontology and Chairman of the Department of Periodontology, Graduate School of Medicine, University of Pennsylvania.

Maury Massler, D.D.S., Professor and Chairman of the Department of Pedodontics, University of Illinois.

Stanley D. Tylman, D.D.S., Professor and Chairman of the Crown and Bridge Department, University of Illinois.

All members and interested dentists and physicians are cordially invited. For reservations and copies of program, please address the Academy Secretary, Dr. Walter I. Levine, 809 Cathedral St., Baltimore, Maryland.

Association of American Women Dentists

The annual meeting of the Association of American Women Dentists will be held in Dallas, Texas, on Monday, Nov. 10, 1958, with headquarters in the Baker Hotel. A suite will be open on Sunday, November 9, for visitors.

Stella Risser, D.D.S.
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Houston 2, Texas

U. S. Department of Health, Education, and Welfare

The Public Health Service orientation film entitled "For the Nation's Health" (16 mm. filmograph, color, sound, 15 minutes, 1957) presents a panoramic view of the activities of the principal health agency of the Federal Government. Combining photographs and motion picture film, it shows the growth of the Public Health Service from its inception in 1798, with limited care of sick and stranded merchant seamen, to its farflung programs today in hospital and medical care, in medical and biological research, and in public health.

Physicians, nurses, dentists, sanitary engineers, pharmacists, veterinarians, and a host of other skilled specialists are shown at work at home and abroad.

In Public Health Service hospitals and clinics, in well-equipped laboratories, on Indian reservations and other field assignments, and on Coast Guard duty, the range and variety of the work of the Public Health Service rarely fails to arouse interest.

Public Health Service personnel have been notified that the film is available to them, principally for training and orientation purposes, through the Communicable Disease Center, Public Health Service, 50 7th St. N.E., Atlanta 5, Georgia.

Others interested and Service personnel also may order the film on a short-term loan from the Surgeon General, Public Health Service (P), Washington 25, D. C. The filmograph may be purchased from Byron, Inc., 1226 Wisconsin Ave., Washington 7, D. C. Cost of the print, $62.07, includes reel can, shipping case, and service charge.

Temple University School of Dentistry

The following course is announced by the Graduate and Postgraduate Division:

Advanced Surgical Techniques in Periodontal Therapy.
April 18-22, 1959. Saul Schluger and Jack Alloy. Tuition, $300.00.

This course is designed to teach and to provide clinical experience with the more recent advances in periodontal surgery. It is intended primarily for the dentist experienced in the basic surgical methods who wishes to extend his range of therapy to include osteoplasty, osteoectomy, extension of the attached gingiva, extension of the vestibular trough, and frenectomy.

Since close supervision is required, the course attendance is necessarily limited to ten. In your application, please give your periodontal background.

For further information and application, write to Director, Graduate and Postgraduate Division, Temple University School of Dentistry, Broad above Allegheny Ave., Philadelphia 40, Pennsylvania.

University of Tennessee Memorial Research Center and Hospital, Department of Dentistry

A straight oral surgery internship approved by the Council on Dental Education of the American Dental Association is offered, to begin Jan. 1, 1959. Training consists of all phases of oral surgery, major and minor, physical and oral diagnosis, radiology, oral pathology, and anesthesiology. The intern attends the Cleft Palate Clinic on the East Tennessee Children's Hospital as a part of his training. The internship runs for twelve months, and an honorarium of $300.00 per month will be paid, plus customary maintenance, laundry, board, and room on premises. Applications must be received by Oct. 15, 1958.

Medical Director
University of Tennessee Memorial Research
Center and Hospital
Alcoa Highway
Knoxville, Tennessee

Tufts University

Tufts University School of Dental Medicine announces the following postgraduate refresher courses:

DPG. 501. Operative and Nutritional Procedures for the Management of Rampant Caries. Nov. 10 and 11, 1958. Frederic Shiere, Professor of Oral Pediatrics, and Abraham Nizel, Instructor in Oral Pathology (Nutrition). Tuition, $35.00.

DPG. 502. Clinical Dentistry for Children. June 15 and 16, 1959. Roland R. Hawes, Eastman Dental Dispensary, Rochester, New York. Tuition, $50.00.

DPG. 801. Oral Roentgenology. Nov. 19, 1958. John Barr, Professor of Oral Diagnosis and Roentgenology. Tuition, $25.00.

DPG. 802. Oral Roentgenology. April 22, 1959. John Barr, Professor of Oral Diagnosis and Roentgenology. Tuition, $25.00.

DPG. 705. Occluso-Rehabilitation I. April 20-24, 1959, 9 A.M. to 5 P.M. Louis Alexander Cohn, Associate Professor of Postgraduate Prosthesis. Tuition, $250.00.

DPG. 901. High-Speed Instruments in Operative Dentistry. Oct. 17 and 18, 1958. Ronald E. R. Lovell and staff. Tuition, $50.00.

DPG. 401. Orthodontic Principles of Interest to the General Practitioner. Eight Wednesdays, Feb. 4 to March 25, 1959. Herbert I. Margolis, Professor and Head of the Department of Orthodontics and Director of Cleft Palate Institute, and staff. Tuition, $150.00.

DPG. 402. Cephalographics and Diagnosis. Jan. 14-16, 1959. Herbert Margolis and staff. Tuition, $100.00.

DPG. 701. Crown and Bridge Prosthesis Participation Course. Jan. 30, 31, Feb. 6, 7, 13, 14, 21, 28, 1959, 9 A.M. to 5 P.M. R. Sheldon Stein, guest postgraduate lecturer. Tuition, $200.00.

DPG. 702. Full Denture Implants. April 6-10, 1959. Norman I. Goldberg, guest postgraduate lecturer, and Aaron Gershkoff, guest postgraduate lecturer. Tuition, $200.00.

DPG. 102. Clinical Endodontics. Nov. 7, 14, 21, 28, Dec. 5 and 12, 1958, 9 A.M. to 5 P.M. Arthur H. Pearson, Associate Professor of Endodontics (Chairman of Department), and staff. Tuition, $150.00.

DPG. 201. Oral Pathology for the Oral Surgeon. Nine Thursdays, Feb. 5 to April 2, 1959, 1 to 4 P.M. Joseph Giammalvo, Assistant Professor of Pathology, Irving Glickman, Professor of Oral Pathology and Periodontology, Gerald Shklar, Assistant Professor of Oral Pathology, and Irving Meyer, Instructor in Oral Surgery and Oral Pathology. Tuition, $150.00.

For further information and application, write to Director, Division of Graduate and Postgraduate Studies, Tufts University School of Dental Medicine, 136 Harrison Ave., Boston, Mass.

University of Alabama

During the month of October, 1958, three refresher courses will be presented:

Root Canal Therapy. Adeeb E. Thomas, D.M.D. October 4, 5, and 6.

Insurance, Investments, and Estate Planning. A. Key Foster, L.L.B., Plato G. Britton, B.S., and Carl P. Heartburg, M.B.A. October 18 and 19.

X-ray Techniques for Dental Hygienists and Assistants. Lincoln R. Manson-Hing, D.M.D. October 24, 25, and 26.

For further information, please write to the Director, Refresher Course Program, University of Alabama School of Dentistry, 1919 Seventh Ave., South, Birmingham, Alabama.

University of Illinois

The Division of Postgraduate Education at the College of Dentistry will offer a concentrated two-week course in oral and maxillofacial surgery beginning Oct. 13, 1958.

Lectures and demonstrations designed to cover all phases of exodontia and oral surgery will be given. Subjects include treatment of cysts, apicoectomy, mouth preparations for dentures, removal of impactions, management of fractures, and care of the hospitalized patient.

Dr. Daniel M. Laskin, associate professor of oral surgery, will direct the course. It will be taught by faculty members of the Colleges of Dentistry and Medicine. The course is planned primarily for dentists in general practice but will not exclude specialists in oral surgery.

Information and applications can be obtained from the Division of Postgraduate Education, University of Illinois College of Dentistry, 808 S. Wood St., Chicago, Illinois.

Temple University School of Dentistry
Graduate and Postgraduate Program for 1958-1959

I. *Anatomy*

Head and Neck Dissection. R. M. Snodgrasse, Ph.B., M.A., Ph.D. Feb. 4 to June 10, 1959 (Wednesdays, 1 to 5 P.M.).

II. *Crown and Bridge*

Fixed Prosthesis, Advanced. G. Straussberg, D.D.S. Jan. 7, 14, 21, and 28, Feb. 4, 11, and 25, March 18 and 25, 1959.

The Porcelain Jacket. J. E. Ewing, D.D.S., and M. J. Kotanchik, D.D.S. Nov. 6 to 8, 1958.

III. *Electrosurgery*

Electronic Electrosurgery. M. Oringer, D.D.S. March 20 and 21, 1959.

IV. *Endodontics*

Clinical Endodontics. L. Parris, D.D.S., and H. Schilder, D.D.S. Oct. 20 to 25, 1958.

Immediate Root Resection. L. Grossman, D.D.S., and L. Parris, D.D.S. June 1 to 6, 1959.

V. *Occlusal Rehabilitation*

Periodontal Prosthesis. M. Amsterdam, D.D.S., and D. W. Cohen, D.D.S. Feb. 16 to 20, 1959.

Gnathology and Oral Rehabilitation. L. Udis, D.D.S., and E. Udis, D.D.S. March 9 to 14, 1959.

Transographics in Full Mouth Restoration. E. J. Hoffman, D.D.S. May 4 to 9, 1959.

VI. *Oral Medicine*

Clinical Oral Pathology. M. Jacobs, D.M.D., M.D. Jan. 15 to 17, 1959.

Oral Medicine for General Practitioner. L. W. Burket, D.D.S., M.D., D.Sc., and S. L. Rosenthal, D.D.S. March 9 to 13, 1959.

VII. *Orthodontics*

Graduate Orthodontics. Robert B. Hedges, D.D.S., M.S. New classes begin in September of each year.

Advanced Orthodontics. Robert H. W. Strang, M.D., D.D.S., Will M. Thompson, D.D.S., and Glenn H. Whitson, D.D.S. Jan. 18 to 31, 1959.

VIII. *Periodontics*

Fundamental Periodontics. Robert Gottsegan, D.D.S. June 1 to 5, 1959.

Advanced Surgical Techniques in Periodontal Therapy. Saul Schluger, D.D.S., and Jack Alloy, D.D.S. April 18 to 22, 1959.

IX. *Practice Administration*

Practice Administration. Jay H. Eshleman, D.D.S., D.Sc. Feb. 28, 1959.

X. *Prosthodontics*

Full Denture Prosthesis. Bernard Jankelson, D.M.D. April 6 to 11, 1959.

Full Denture Prosthesis, Advanced. Bernard Jankelson, D.M.D. April 12 to 18, 1959.

XI. *Radiodontics*

Radiodontics, Advanced. William J. Updegrave, D.D.S. Jan. 22 to 24, 1959.

XII. *Special*

Special full-year postgraduate course. Registration date: Sept. 11, 1958, 9 A.M.

Time, Motion and Speed. E. O. Thompson, D.D.S. March 1 to 3, 1959.

For additional information and application, write to Temple University School of Dentistry, 3223 North Broad St., Philadelphia 40, Pennsylvania.

University of Washington

The School of Dentistry offers a new course of graduate study in periodontics. The course is of eighteen months' duration and, upon the satisfactory completion of a thesis in addition to the acceptable performance of the remainder of the academic program, leads to the granting of a Master of Science degree in dentistry.

The course will be directed toward the training of teachers and research workers in periodontics and allied fields. Extensive clinical training will be offered, in addition to the projection of a basic science background.

Inquiries should be addressed to Saul Schluger, D.D.S., Director Graduate Dental Education, University of Washington, School of Dentistry, Seattle 5, Washington.

Deutsche Gesellschaft für Kiefer- und Gesichtschirurgie

The eighth meeting of the German Society for Surgery of the Jaws and Face will be held Aug. 28 to 30, 1958, at Hamburg, Germany, in the University Hospital, Eppendorf. The preliminary program is divided into four parts:

1. Prevention and Treatment of Pain in the Jaws and Face—General Anesthesia, Local Anesthesia, and Pain Therapy.
2. Treatment of Lip and Palate Clefts.
3. Newer Methods for the Treatment of Fractures of the Facial Region.
4. Free presentations of subjects to be announced.

A social evening will be held August 29 at the Hotel Atlantik.

For further information and applications for participation, write to Prof. Karl Schuchadt, Johnsalle 3, Hamburg 13, Germany.

REVIEWS OF NEW BOOKS

Clinical Periodontology: The Periodontium in Health and Disease (Recognition, Diagnosis and Treatment of Periodontal Disease in the Practice of General Dentistry). By Irving Glickman, B.S., D.M.D., F.A.C.D., Professor of Oral Pathology and Periodontology and Director of the Division of Graduate and Postgraduate Studies, Tufts University School of Dental Medicine. Philadelphia and London, W. B. Saunders Company. Price, $17.50. 978 pages. 720 illustrations.

This is the second edition by Glickman, who is well known and appreciated in the field of periodontology.

In general, the text has been revised—in particular, the section which deals with the clinical management of periodontal disease.

The references at the end of each chapter have been brought up to date.

THOMAS J. COOK

A Textbook of Oral Pathology. By William G. Shafer, B.S., D.D.S., M.S., Associate Professor of Oral Pathology, Indiana University School of Dentistry; Maynard K. Hine, D.D.S., M.S., Dean, Professor of Periodontology, Indiana University School of Dentistry; and Barnet M. Levy, A.B., D.D.S., M.S., Professor of Pathology, University of Texas Dental Branch. Philadeplhia and London, W. B. Saunders Company. Price, $15.00.

This text is made up in five sections: (I) Disturbances of Development and Growth, (II) Diseases of Microbial Origin, (III) Injuries and Repair, (IV) Disturbances of Metabolism, and (V) Diseases of Specific Systems. Each section is divided into chapters, of which there are seventeen. The photographs of clinical subjects and roentgenograms are excellent. The photomicrographs are well placed, and the interpretation is adequate. There are references at the end of each chapter which should aid those interested in additional reading.

This monograph is recommended to the dental student and practitioner.

THOMAS J. COOK